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Quantitative Labeling Using Tandem Mass Tags

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Omics marketing group
September 13, 2018

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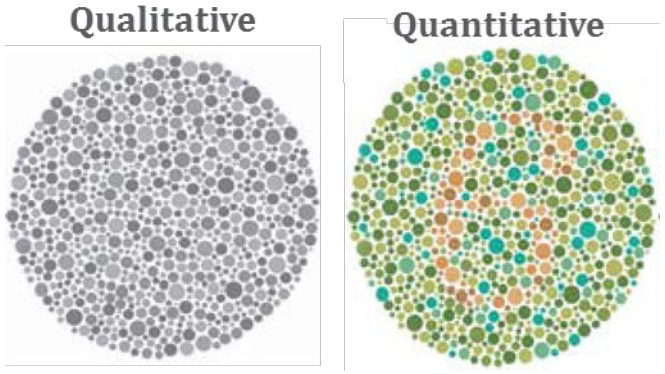
Introduction: TMT based relative quantification

Moving Beyond Qualitative Proteomics

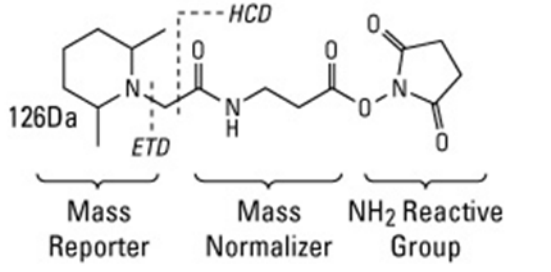
Problem: Quantitative information about expression level of a protein is essential to understanding its biological role in response to change or disease.

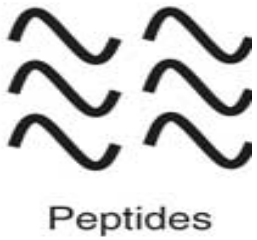


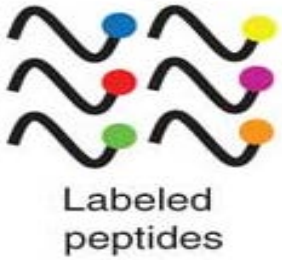
Add another dimension to any experiment by determining the relative abundance of each identified protein

Isobaric Labeling/Tandem Mass Tags™ (TMT)*

TMTsixplex™







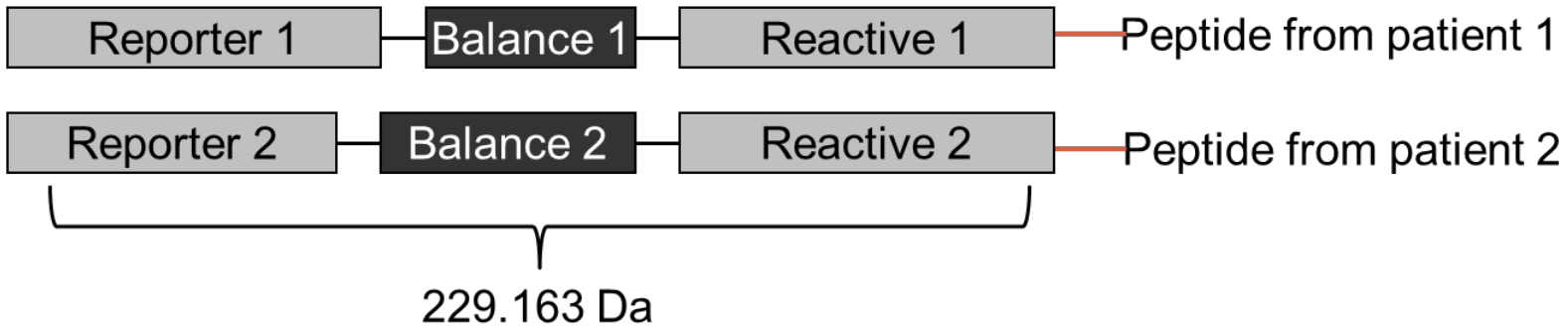
LC-MS2

One signal

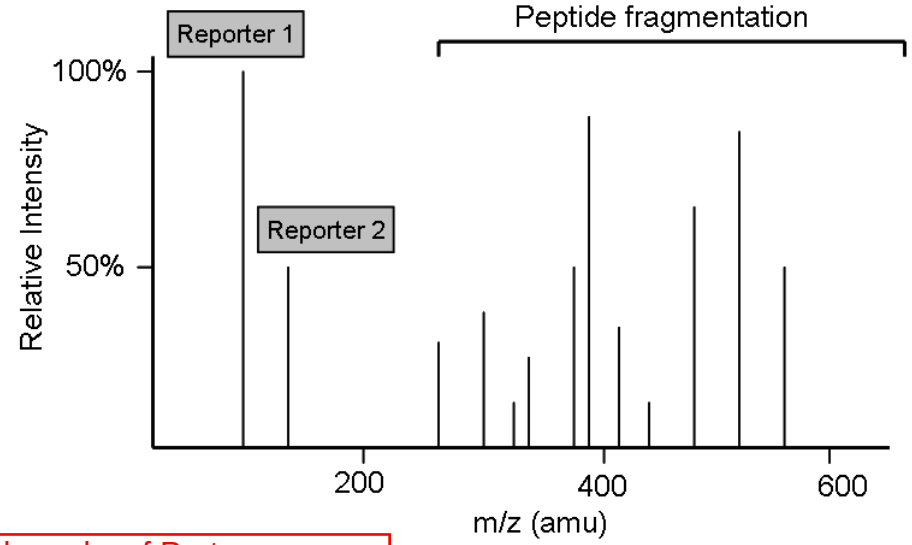
HCD fragmentation

* Ting, L. et al. 2011. Nature Methods 8: 937-940

How Does Isobaric Mass Tagging (TMT&iTRAQ) Work?

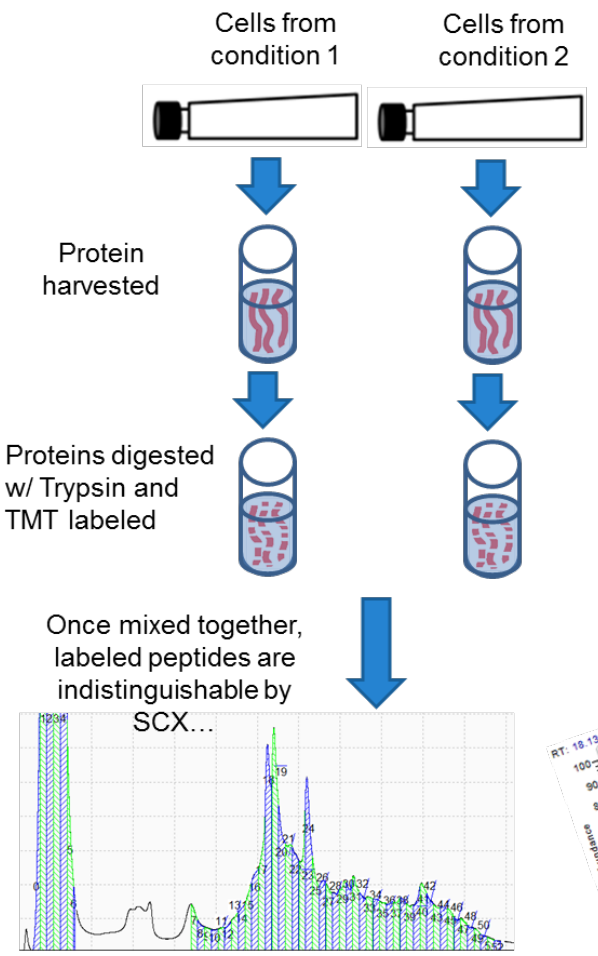


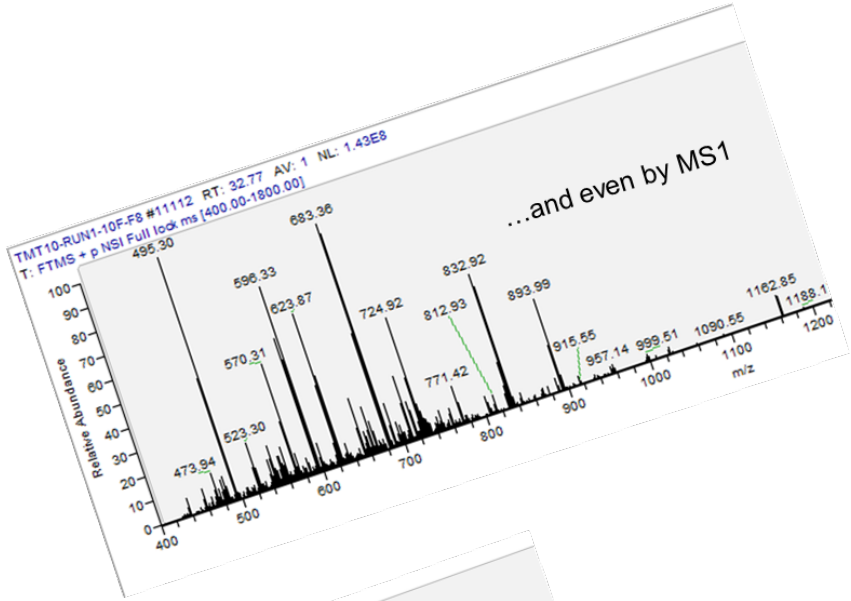
The tagged peptides behave exactly the same, except during fragmentation.

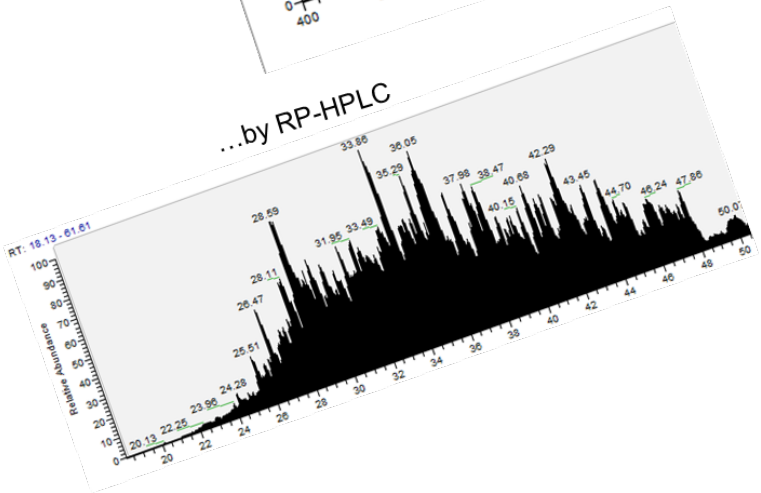


This spectra indicates that this protein is upregulated in patient 1 approximately 2 fold

TMT Labels are Indistinguishable







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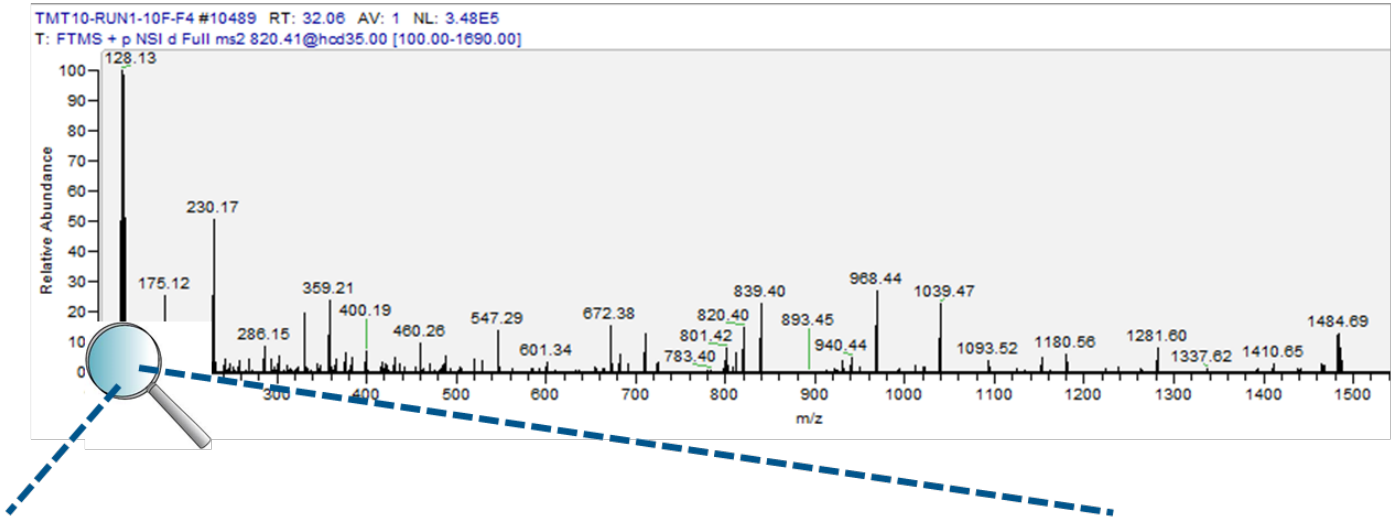
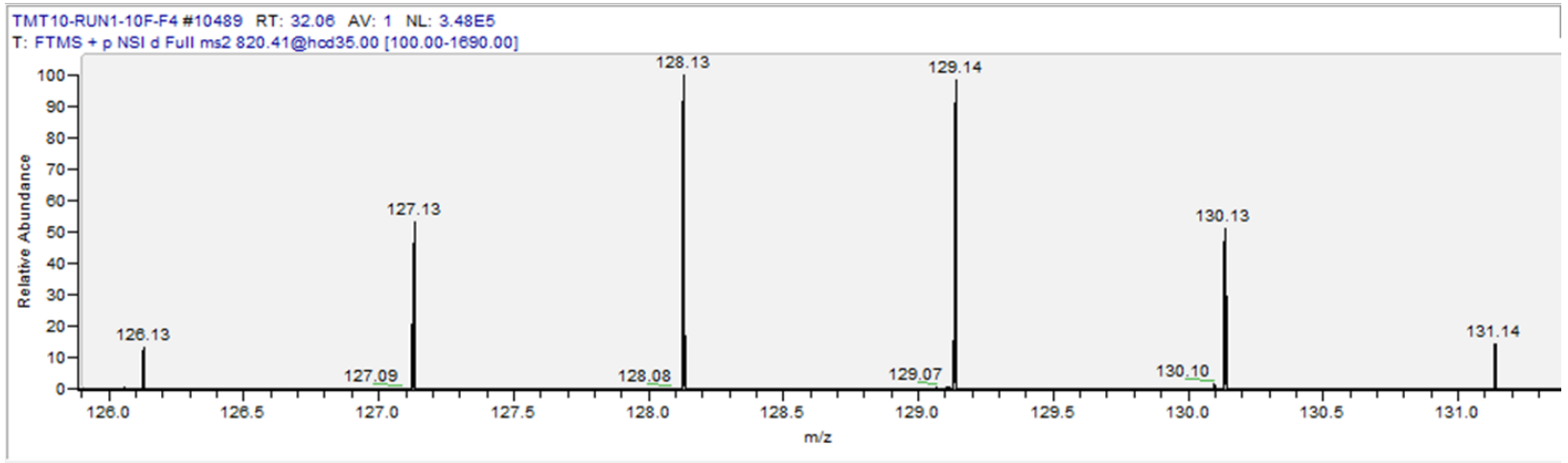
The Difference Only Appears in MSn





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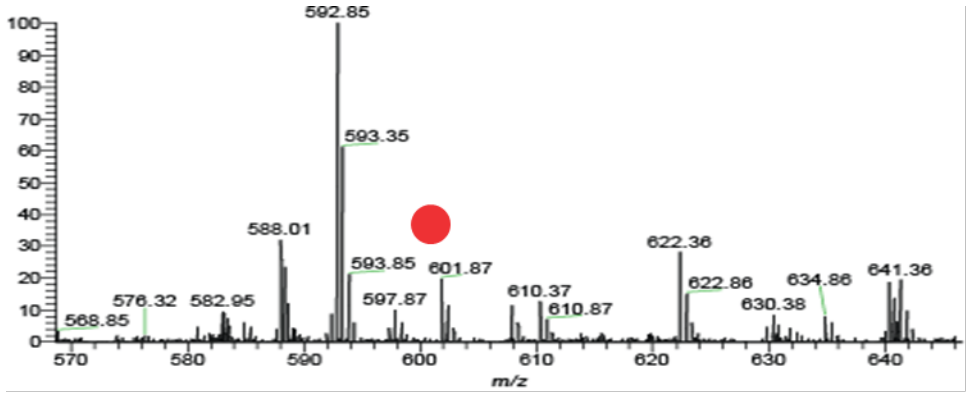
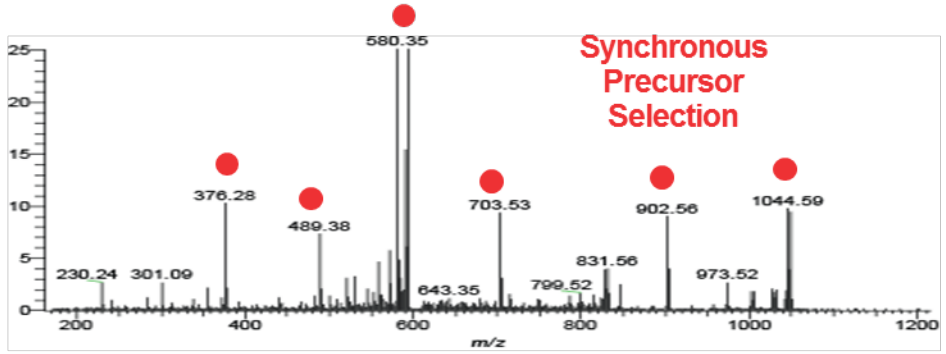
Synchronous Precursor Selection (SPS) for Accurate Quantification

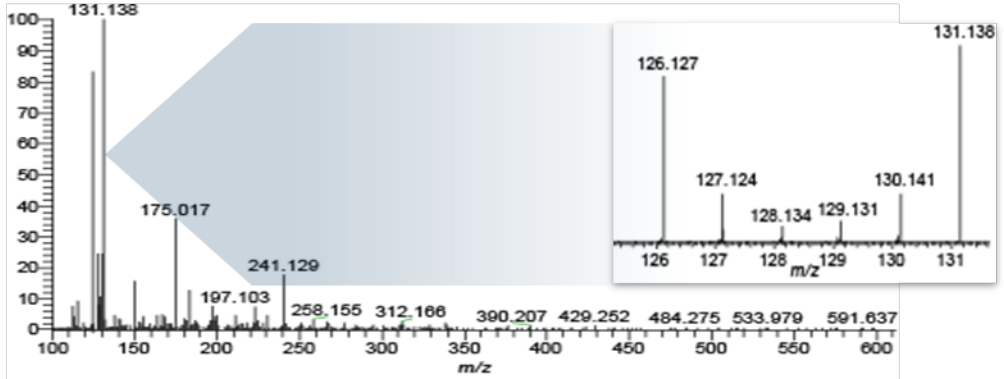
Precursor Ion



CID MS^2, Ion Trap



HCD MS^3, Orbitrap



- Available on the Thermo Scientific™ Orbitrap Fusion™ MS and Thermo Scientific™ Orbitrap Fusion™ Lumos ™ MS
- Select multiple MS^2 precursors using a single fill and notched isolation waveform
- Improves the ratio accuracy and at the same time dramatically boosts sensitivity

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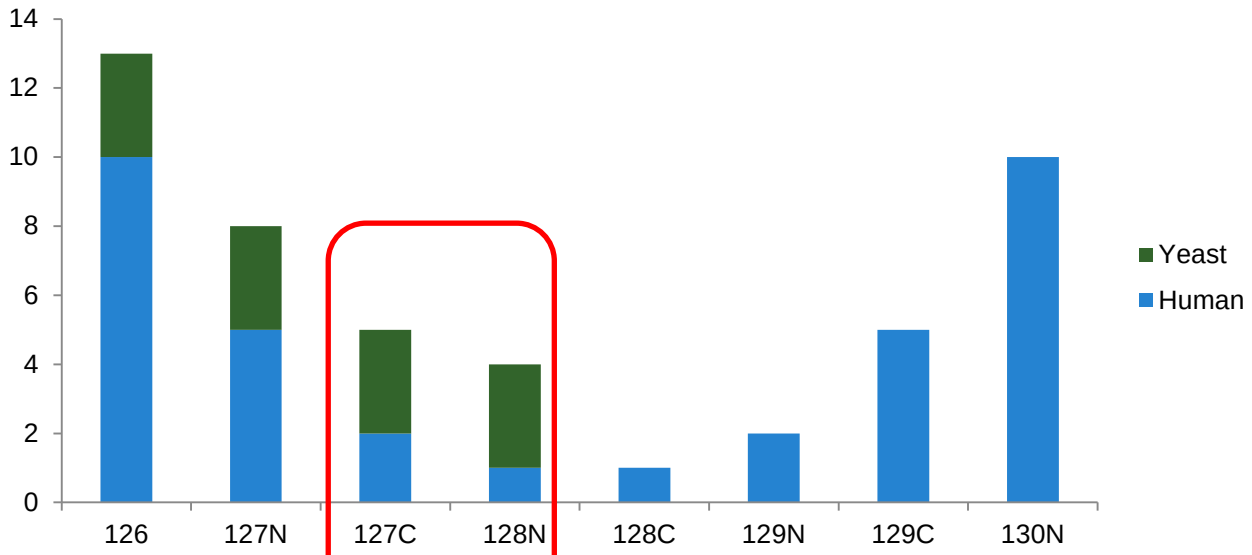
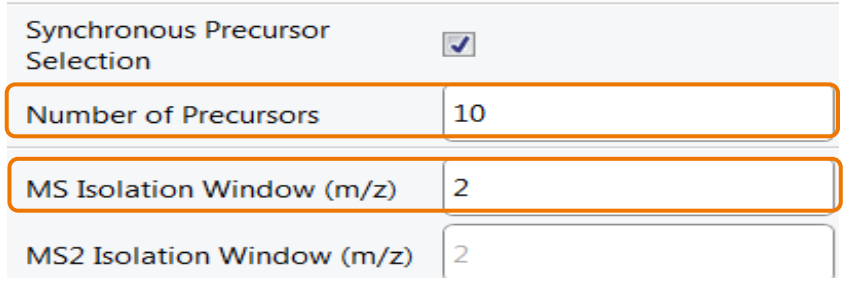
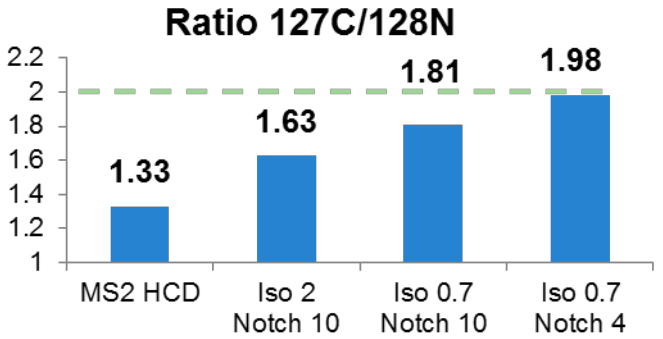
Multinotch MS^3 Quantification is Accurate and Sensitive

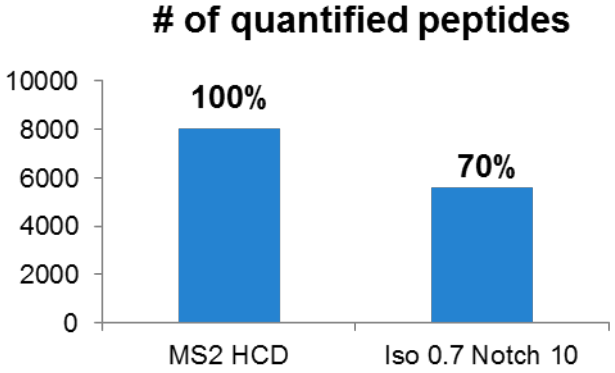


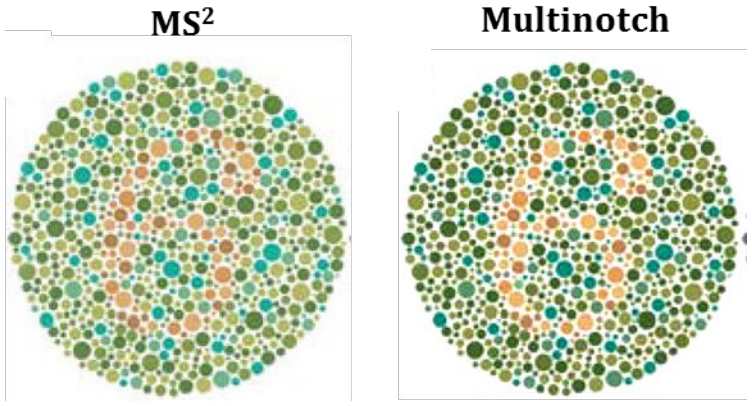
Human 10 : 5 : 2 : 1 : 1 : 2 : 5 : 10

Yeast 1 : 1 : 1 : 1









* TMT MS^3 Peptides-Quan template in Method Editor

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TMT11plex

Reporter Ion Mass			Reporter Ion Mass
	TMT10-126		126.1277261
127.1247610	TMT10-127N	TMT10-127C	127.1310809
128.1281158	TMT10-128N	TMT10-128C	128.1344357
129.1314706	TMT10-129N	TMT10-129C	129.1377905
130.1348254	TMT10-130N	TMT10-130C	130.1411453
131.1381802	TMT10-131	TMT11-131C	131.144999

- TMT11-131C can be used in combination with TMT10plex reagents to multiplex 11 different samples for MS analysis
- 11plex data analysis is supported by Proteome Discoverer 2.1-2.3

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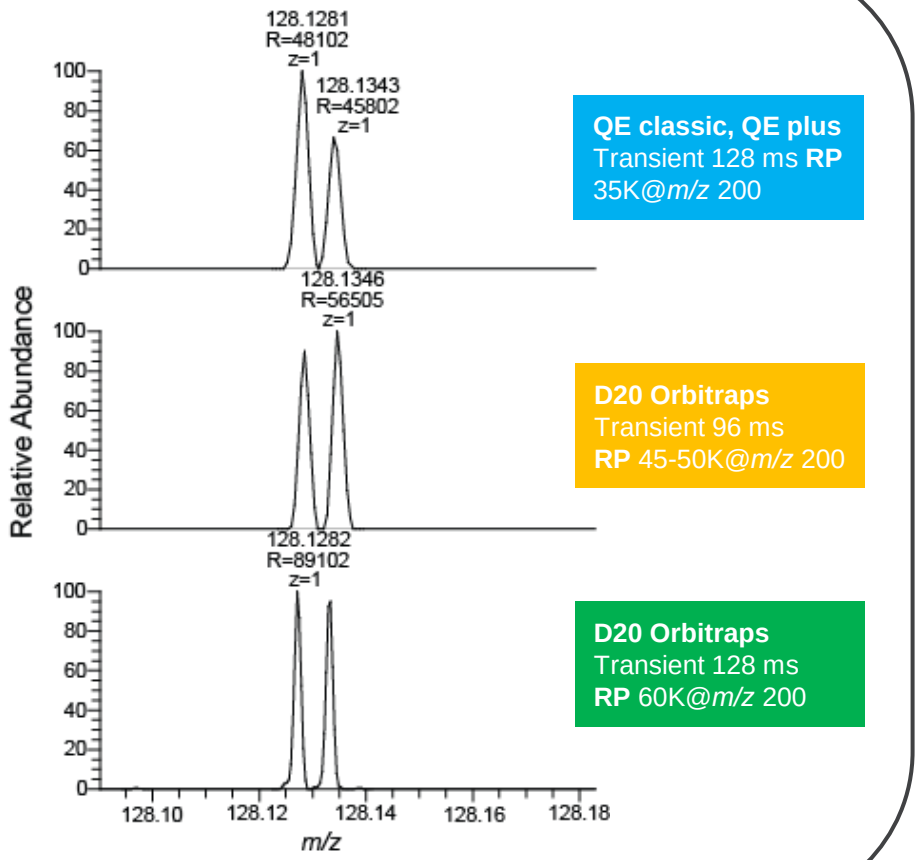
High Resolving Power is Required for Accurate Quantification of the TMT11 plex



QE classic, QE plus
Transient 128 ms **RP** 35K@*m/z* 200

D20 Orbitraps
Transient 96 ms
RP 45-50K@*m/z* 200

D20 Orbitraps
Transient 128 ms
RP 60K@*m/z* 200

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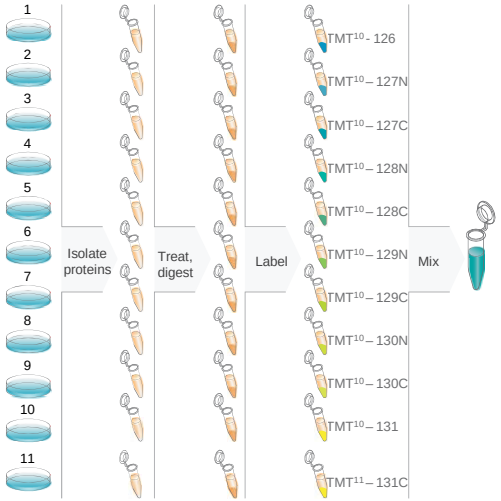
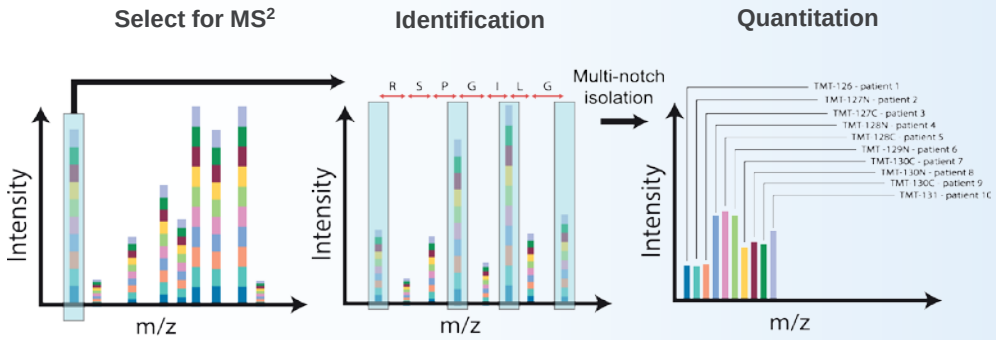
TMT Multiplexing Workflow for Precise Data in Less Time

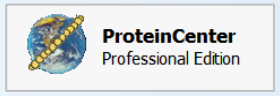




Sample Labeling and Preparation → **LC-MS/MS (SPS MS3) Analysis** → **Data Analysis**

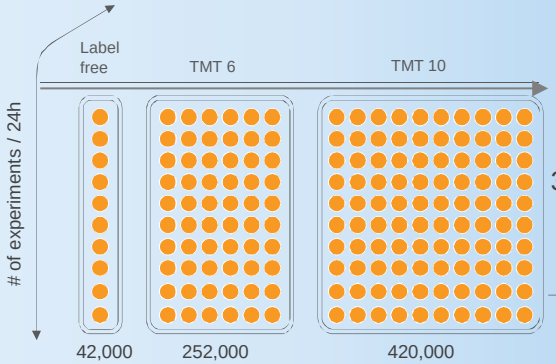
Thermo Scientific™ TMT™ 11-Plex Reagents

Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ MS with Method Templates



Thermo Scientific™ Proteome Discoverer™ Software and ProteinCenter™ Software

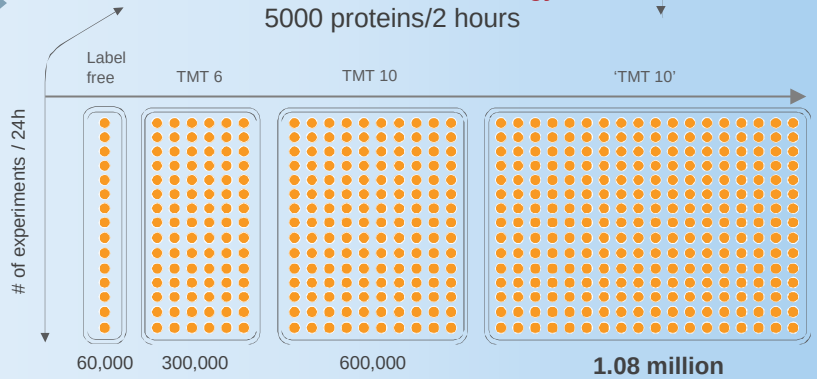
Number of protein quantifications per day



Current Technology
3500 proteins/2 hours

Advances in Tribrid MS technology

Next Generation Technology
5000 proteins/2 hours



Unique workflow with potential for massive throughput – *improvements in instrument technology*

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TMT QC assay - TMT 11 yeast triple knock out standard

TMT Standard for TMT Workflow QC and Method Development

- **MS and LC method optimization**
- **QC of mass spec and LC**
- **PD analysis optimization**

"TMT standard will not solve the interference problem, it can accurately and sensitively measure its effects"

J.Paulo et al, JASMAS, 2016, 1620-1625

TMT QC Assay Standard

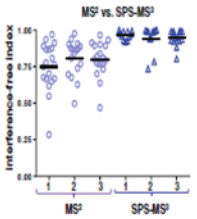
J. Am. Soc. Mass Spectrom. (2016)
DOI: 10.1007/s13361-016-1434-9

RESEARCH ARTICLE

A Triple Knockout (TKO) Proteomics Standard for Diagnosing Ion Interference in Isobaric Labeling Experiments

Joao A. Paulo, Jeremy D. O'Connell, Steven P. Gygi

Department of Cell Biology, Harvard Medical School, Boston, MA 02115, USA



Abstract. Isobaric labeling is a powerful strategy for quantitative mass spectrometry-based proteomic investigations. A complication of such analyses has been the co-isolation of multiple analytes of similar mass-to-charge resulting in the distortion of relative protein abundance measurements across samples. When properly implemented, synchronous precursor selection and triple-stage mass spectrometry (SPS-MS3) can reduce the occurrence of this phenomenon, referred to as ion interference. However, no diagnostic tool is available currently to rapidly and accurately assess ion interference. To address this need, we developed a multiplexed tandem mass tag (TMT)-based standard, termed the triple knockout (TKO). This standard is comprised of three yeast proteomes in triplicate, each from a strain deficient in a highly abundant protein (Met6, Pfk2, or Ura2). The relative abundance patterns of these proteins, which can be inferred from dozens of peptide measurements can demonstrate ion interference in peptide quantification. We expect no signal in channels where the protein is knocked out, permitting maximum sensitivity for measurements of ion interference against a null background. Here, we emphasize the need to investigate further ion interference-generated ratio distortion and promote the TKO standard as a tool to investigate such issues.

Keywords: MS standard, MuiltiNotch, TMT, Orbitrap Fusion, Lumos, Ion interference, SPS-MS3

Received: 12 April 2016/Revised: 30 May 2016/Accepted: 31 May 2016

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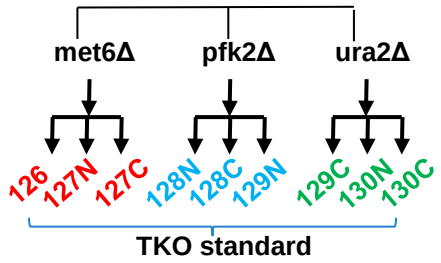
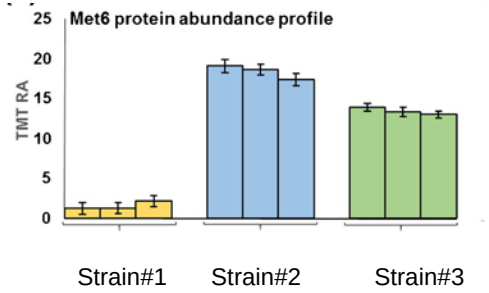
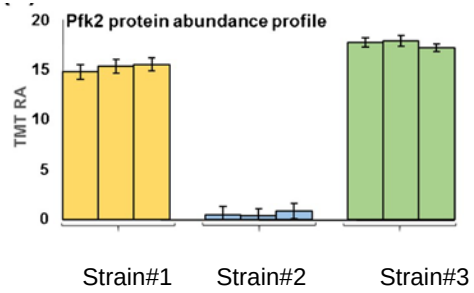
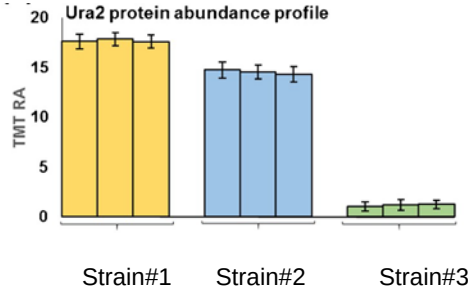
Difference between Proteomics TKO Standard and Pierce TMT TKO standard

- TKO standard (Paulo et al, 2016)
  - Paulo's TKO standard uses met6(protein rank19), pfk2(114) and ura2(244) yeast strains.
  - Paulo et al uses TMT9 to label peptides from those three strains.

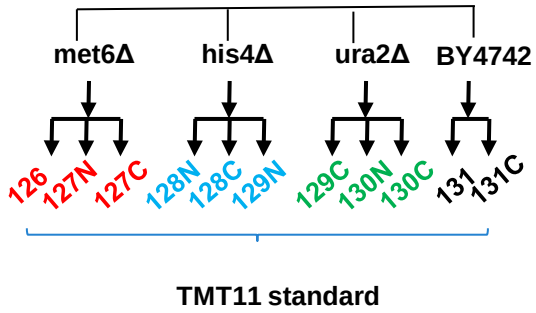




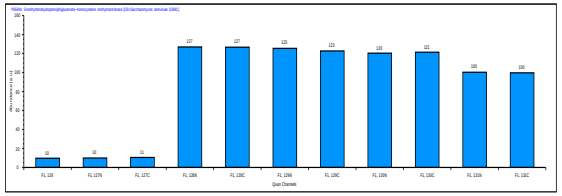




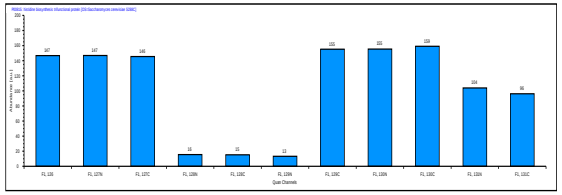
- Pierce TMT11 TKO Standard
  - Pierce TMT11 standard uses met6,his4(213),ura2(244), and BY4742 parental yeast strains.
  - The standard uses BY4742 parental strain labeled with 131N and 131C as control channels.



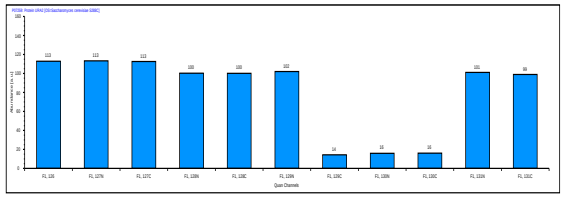
Met6 protein abundance profile



His4 protein abundance profile



Ura2 protein abundance profile



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Experimental Set up

TMT11 TKO Sample Reconstitution

- Take vial out of the freezer and bring to the room temperature, 15 min
- Add 40 ul of 0.1%TFA/5% AcN in Water, 500 ng/ul
- Or add 40 ul of 5% DMSO/1% FA in Water, 500 ng/ul
- Incubate at RT for 15 min and transfer to autosampler vial
- Don’t keep more than 1 week at 4 C

LC Method: EASY-Spray™ C18 50cm column & UltiMate™ 3000 RSLCnano UHPLC

50 min gradient

No	Time	Flow [µl/min]	%B	Curve
1	0.000		Equilibration	
2	0.000	0.300	2.0	5
3	*New Row*			
4	0.000		Run	
5	14.000	0.300	2.0	5
6	17.000	0.300	4.0	5
7	67.000	0.300	28.0	5
8	70.000	0.300	85.0	5
9	75.000	0.300	85.0	5
10	77.000	0.300	4.0	5
11	*New Row*			
12	100.000		Stop Run	

120 min gradient

No	Time	Flow [µl/min]	%B	Curve
1	0.000		Equilibration	
2	0.000	0.300	2.0	5
3	*New Row*			
4	0.000		Run	
5	14.000	0.300	2.0	5
6	17.000	0.300	4.0	5
7	100.000	0.300	16.0	5
8	145.000	0.300	25.0	5
9	150.000	0.300	85.0	5
10	158.000	0.300	85.0	5
11	160.000	0.300	4.0	5
12	*New Row*			
13	185.000		Stop Run	

Solvent A:	0.1% formic acid
Solvent B:	100 % Acetonitrile, 0,1% formic acid
Flow rate:	300 nL/min
Injection volume:	1-2 µL

Direct injection

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LC Method: EASY-Spray™ C18 50cm column & UltiMate™ 3000 RSLCnano UHPLC

Loading pump conditions

Time(min)	Flow, ul/min
0	20
100, 185	20

50 min gradient

No	Time	Flow [µl/min]	%B	Curve
1	0.000	Equilibration		
2	0.000	0.300	2.0	5
3	*New Row*			
4	0.000	Run		
5	14.000	0.300	2.0	5
6	17.000	0.300	4.0	5
7	67.000	0.300	28.0	5
8	70.000	0.300	85.0	5
9	75.000	0.300	85.0	5
10	77.000	0.300	4.0	5
11	*New Row*			
12	100.000	Stop Run		

120 min gradient

No	Time	Flow [µl/min]	%B	Curve
1	0.000	Equilibration		
2	0.000	0.300	2.0	5
3	*New Row*			
4	0.000	Run		
5	14.000	0.300	2.0	5
6	17.000	0.300	4.0	5
7	100.000	0.300	16.0	5
8	145.000	0.300	25.0	5
9	150.000	0.300	85.0	5
10	158.000	0.300	85.0	5
11	160.000	0.300	4.0	5
12	*New Row*			
13	185.000	Stop Run		

Solvent A: 0.1% formic acid

Solvent B: 100 % Acetonitrile, 0,1% formic acid

Flow rate: 300 nL/min

Injection volume: 1-2 µL

Trap loading

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LC Method using EASY-Spray™ C18 50cm column & EASY-nLC™ 1200 HPLC

50 min

Time (min)	Flow(nL/min)	%B
0	300	5
5	300	10
55	300	40
60	300	90
70	300	90

120 min

Time (min)	Flow(nL/min)	%B
0	300	5
5	300	8
125	300	40
130	300	90
140	300	90

Solvent A:	0.1% formic acid
Solvent B:	80 % Acetonitrile, 0,1% formic acid
Flow rate:	300 nL/min
Injection volume:	1-2 µL

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LC Method using EASY-Spray™ C18 50cm column & EASY-nLC™ 1000 HPLC

50 min

Time (min)	Flow(nL/ min)	%B
0	300	5
5	300	7
55	300	32
60	300	90
70	300	90

120 min

Time (min)	Flow(nL/min)	%B
0	300	5
5	300	7
125	300	32
130	300	90
140	300	90

Solvent A:	0.1% formic acid
Solvent B:	100 % Acetonitrile, 0,1% formic acid
Flow rate:	300 nL/min
Injection volume:	1 µL

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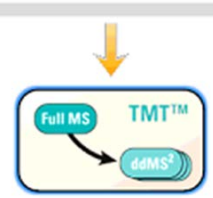
Instrument Method Settings: QExactive Classic and QExactive Plus

Properties	QE classic 50 min	QE classic 120 min	QE + 50 min	QE + 120 min
Resolution Full MS	70000	70000	70000	70000
AGC target Full MS	3e6	3e6	3e6	3e6
MS max IT, ms	50	50	50	50
Scan range, *m/z*	350-1500	350-1500	350-1500	350-1500
Loop count	15	15	15	15
MS2 resolution	35000	35000	35000	35000
MS2 AGC target	1e5	1e5	1e5	1e5
MS2 max IT, ms	120 ms	250 ms	100 ms	120 ms
Isolation Window , Th	1.2	1.2	0.7	0.7
NCE, %	32-34	32-34	32-34	32-34
Intensity threshold	1e4	1e4	1e4	1e4
Peptide match	preferred	preferred	preferred	preferred
Dynamic exclusion, s	20 s	45 s	20 s	30 s
First mass, *m/z*	110	110	110	110

Instrument Method Settings: QExactive HF and QExactive HF X

Properties	QE HF 50 min	QE HF 120 min	QE HF X 50 min	QE HF X 120 min
Resolution Full MS	120000	120,000	120000	120000
AGC target Full MS	3e6	3e6	3e6	3e6
MS max IT, ms	50	50	50	50
Scan range, m/z	350-1500	350-1500	350-1500	350-1500
Loop count	20	15	20	15
MS2 resolution	60000(2.9-45000)	60000(2.9- 45000)	45000	45000
MS2 target	1e5	1e5	1e5	1e5
MS2 max IT, ms	96	120	86	96
Isolation Window, Th	0.4 m/z	0.7 m/z	0.7 m/z	0.7 m/z
NCE, %	32-34	32-34	32-34	32-34
Intensity threshold	1e4	1e4	1e4	1e4
Peptide match	preferred	preferred	preferred , single charge	preferred , single charge
Dynamic exclusion, s	20	30	20	30
First mass, m/z	110	110	110	110



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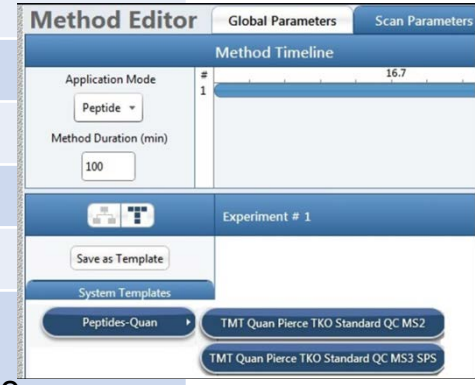
Instrument Method Settings: Fusion, Tune3.1 Templates are Available in Method Editor

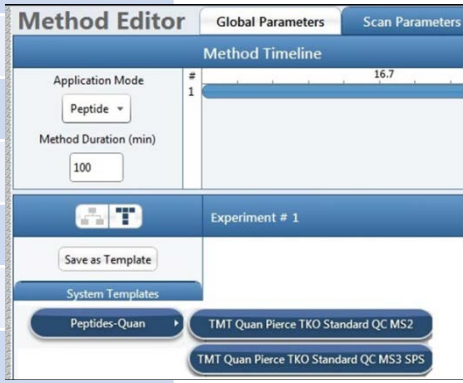
Properties	Fusion SPS 50 min	Fusion SPS 120 min	Fusion MS2 50 min	Fusion MS2 120 min
Resolution Full MS	120000	120000	120000	120000
AGC target Full MS	4e5	4e5	4e5	4e5
MS max IT, ms	50	50	50	50
Scan range, m/z	375-1500	375-1500	375-1500	375-1500
Top Speed, s	2	3	2	3
MS2 max IT, ms	50	50	105	120
MS2 Isolation window, Th	1.2(2)-0.7(3)-0.5 (4+)	1.2(2)-0.7(3)-0.5 (4+)	1.2(2)-0.7(3)-0.5 (4+)	0.7(2-3)-0.5 (4+)
MS2 NCE, %	35	35	38-40	38-40
MS2 Intensity threshold	5e3	5e3	5e4	5e4
Dynamic exclusion, s	45, single charge	60, single charge	45, single charge	60, single charge
MS2 Resolution	turbo	turbo	50000	50000
MS2 AGC target	1e4	1e4	1e5	1e5
MS3 AGC target	1e5	1e5		
SPS Isolation window, Th	1.3(2)-0.7(3)-0.5 (4+)	1.3(2)-0.7(3)-0.5 (4+)		
SPS NCE, %	65	65		
SPS max IT, ms	105	120		
SPS settings: # notches, mass range	5-10-10 *m/z* 110-500	5-10-10 *m/z* 110-500	*m/z* 110	*m/z* 110



ThermoFisher SCIENTIFIC

Instrument Method Settings: Lumos, Tune3.1 Templates are Available in Method Editor

Properties	Fusion SPS 50 min	Fusion SPS 120 min	Fusion MS2 50 min	Fusion MS2 120 min
Resolution Full MS	120000	120000	120000	120000
AGC target Full MS	4e5	4e5	4e5	4e5
MS max IT, ms	50	50	50	50
Scan range, m/z	375-1500	375-1500	375-1500	375-1500
Top Speed, s	2	3	2	3
MS2 max IT, ms	50	50	86	96
MS2 Isolation window, Th	1.2(2)-0.7(3)-0.5 (4+)	1.2(2)-0.7(3)-0.5 (4+)	1.2(2)-0.7(3)-0.5 (4+)	0.7(2-3)-0.5 (4+)
MS2 NCE, %	35	35	38-40	38-40
MS2 Intensity threshold	5e3	5e3	5e4	5e4
Dynamic exclusion, s	45, single charge	60, single charge	45, single charge	60, single charge
MS2 Resolution	turbo	turbo	50000	50000
MS2 AGC target	1e4	1e4	1e5	1e5
MS3 AGC target	1e5	1e5		
SPS Isolation window, Th	1.3(2)-0.7(3)-0.5 (4+)	1.3(2)-0.7(3)-0.5 (4+)		
SPS NCE, %	65	65		
SPS max IT, ms	86	105		
SPS settings: # notches, mass range	5-10-10 *m/z* 110-500	5-10-10 *m/z* 110-500	*m/z* 110	*m/z* 110



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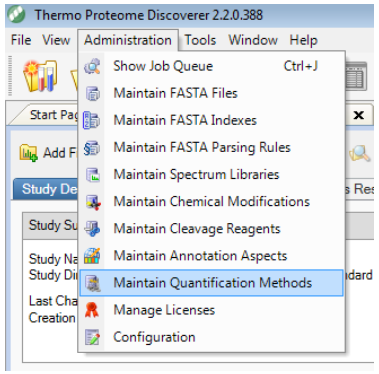
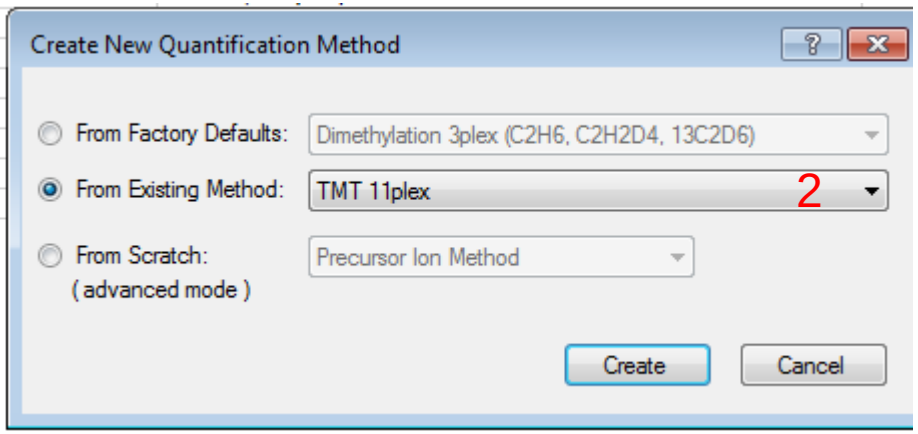
PD 2.2 workflow set up

I. Quantification Method Add Lot Specific Correction Factors



1



Mass Tag	Reporter Ion	-2	-1	Monoisotopic	+1	+2
TMT^{10}-126	126.127726	0%	0%	100%	8.5%	0.3%
TMT^{10}-127N	127.124761	0%	0.5%	100%	8.5%	0.3%
TMT^{10}-127C	127.131081	0%	0.5%	100%	7.3%	0.4%
TMT^{10}-128N	128.128116	0%	0.6%	100%	7.2%	0.2%
TMT^{10}-128C	128.134436	0%	1.3%	100%	6.3%	0.2%
TMT^{10}-129N	129.131471	0%	1.6%	100%	6.2%	0%
TMT^{10}-129C	129.137790	0%	2.5%	100%	5.0%	0.1%
TMT^{10}-130N	130.134825	0%	2.7%	100%	5.0%	0%
TMT^{10}-130C	130.141145	0.1%	2.9%	100%	4.0%	0%
TMT^{10}-131	131.138180	0.1%	3.1%	100%	3.9%	0%

3

4

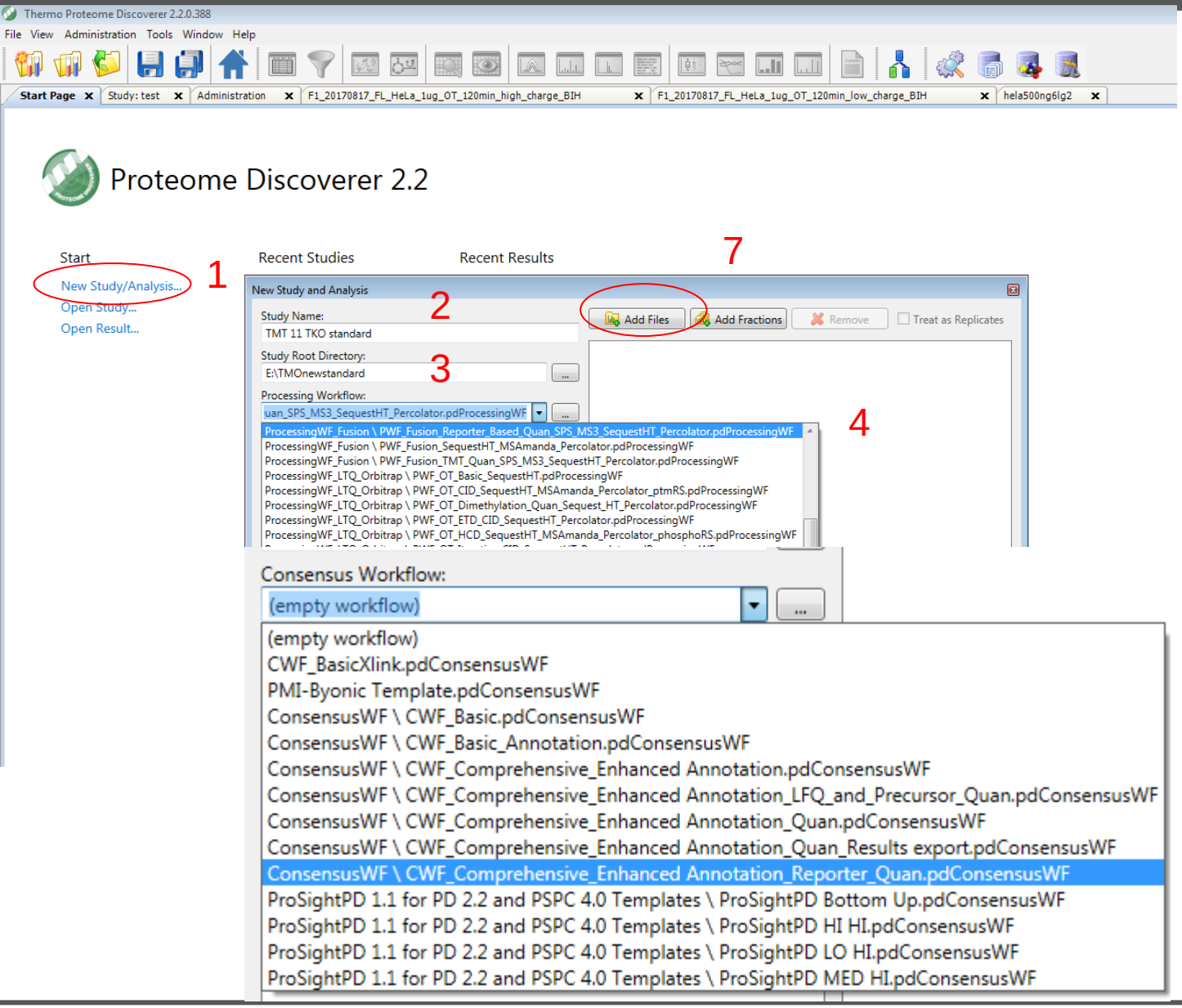
Mass Tag	Reporter Ion Mass	- 2	- 1	Main	+ 1	+ 2	Active
126	126.127726	0	0	100	8.5	0.3	☑
127N	127.124761	0	0.5	100	8.5	0.3	☑
127C	127.131081	0	0.5	100	7.3	0.4	☑
128N	128.128116	0	0.6	100	7.2	0.2	☑
128C	128.134436	0	1.3	100	6.3	0.2	☑
129N	129.131471	0	1.6	100	6.2	0	☑
129C	129.13779	0	2.5	100	5	0.1	☑
130N	130.134825	0	2.7	100	5	0	☑
130C	130.141145	0	2.9	100	4	0	☑
131N	131.13818	0	3.1	100	3.9	0	☑
131C	131.144499	0	1.4	100	2.9	0	☑

TMT: Main peaks are always 100%

OK Cancel Help

1. Select “Maintain Quantification Methods”
2. Create new method using TMT 11plex template
3. Add Correction factors from Product data sheet
4. Save new method as TMT11TKOlotXXX standard

II. Study Set up



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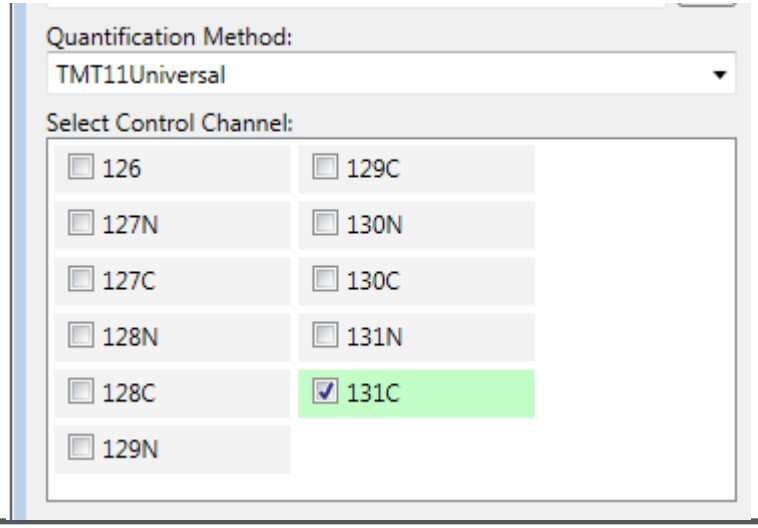

1. Select New Study
2. Create Study Name
3. Select Study Directory
4. Select Processing workflow
5. Select Consensus workflow
6. Select Quan.method and control channel
7. Add files

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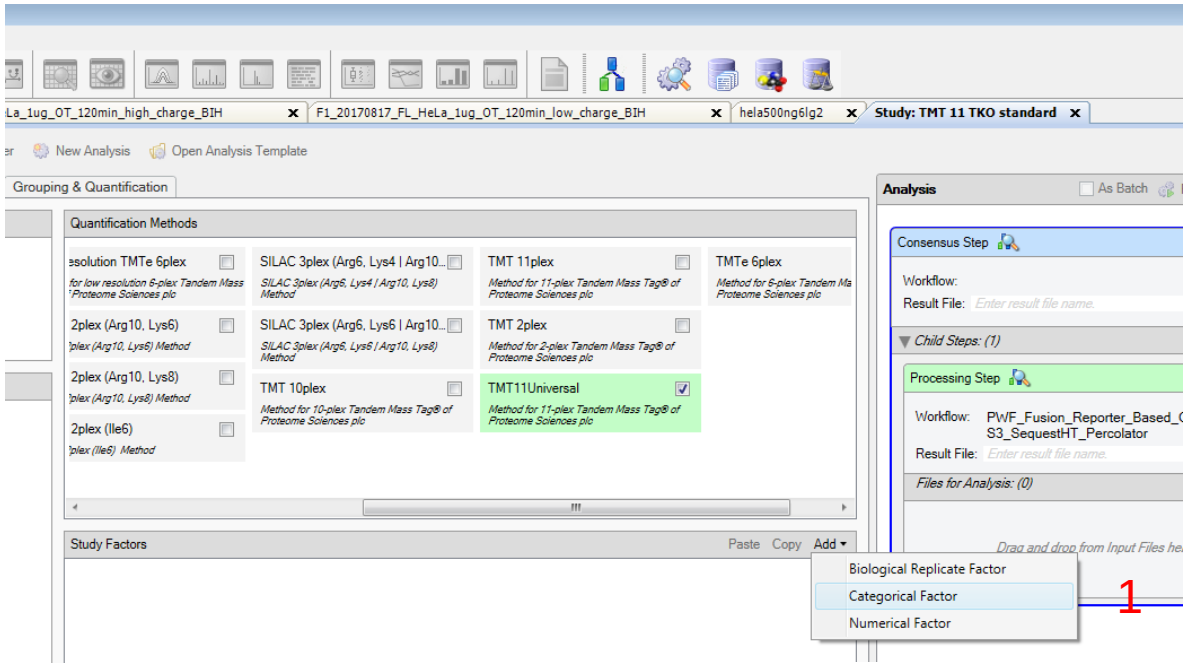
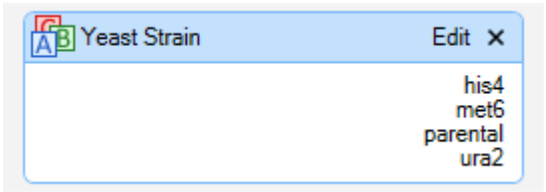
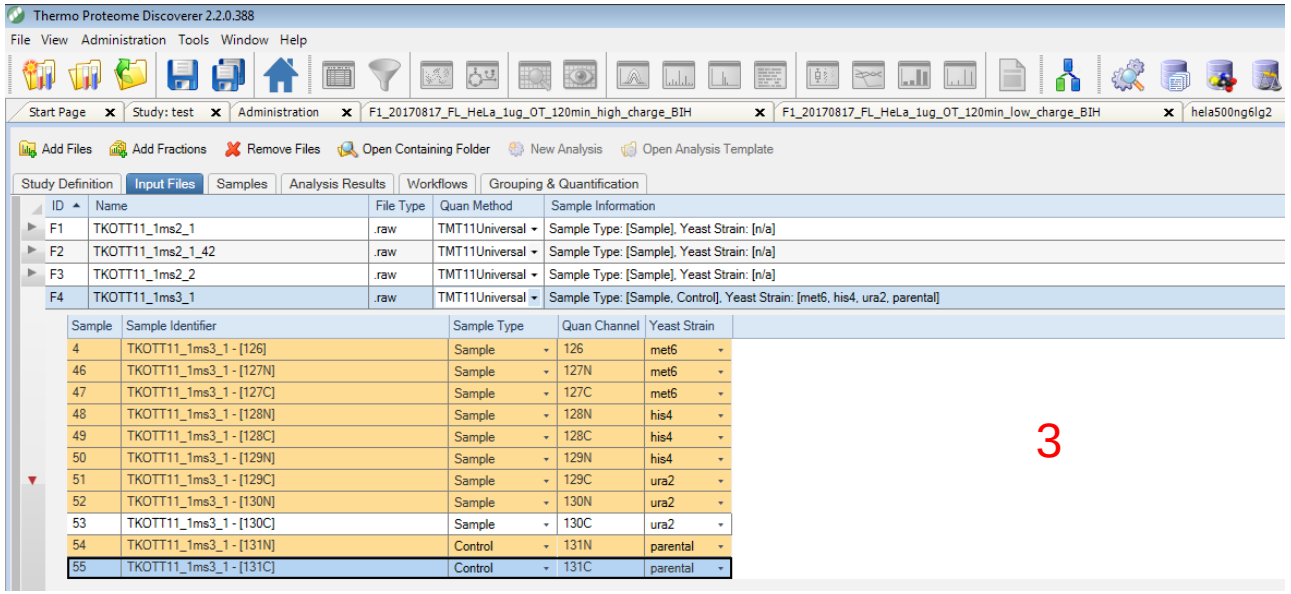
II. Study Set up

1. Select categorical factor
2. Create study factors
3. Set study factors, controls for each of the quan.channels per file

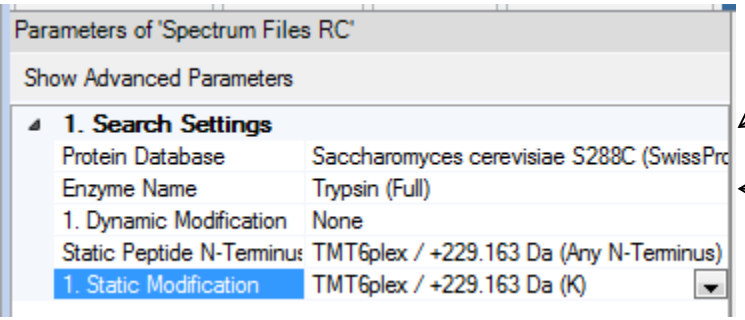


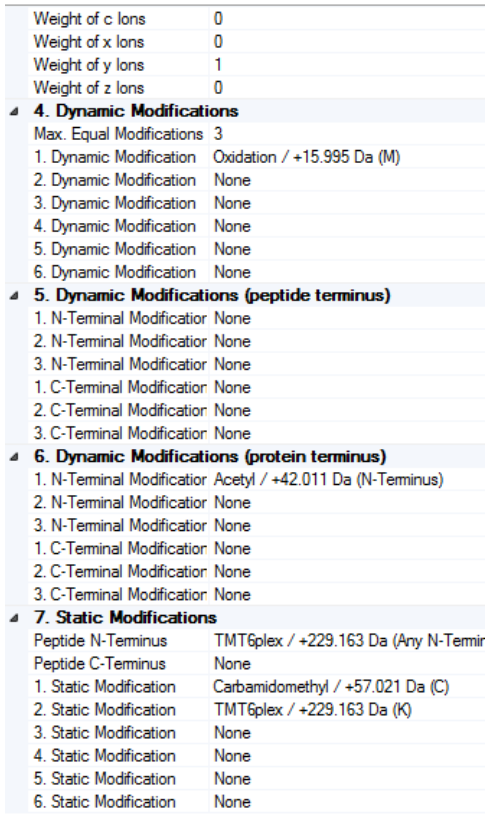


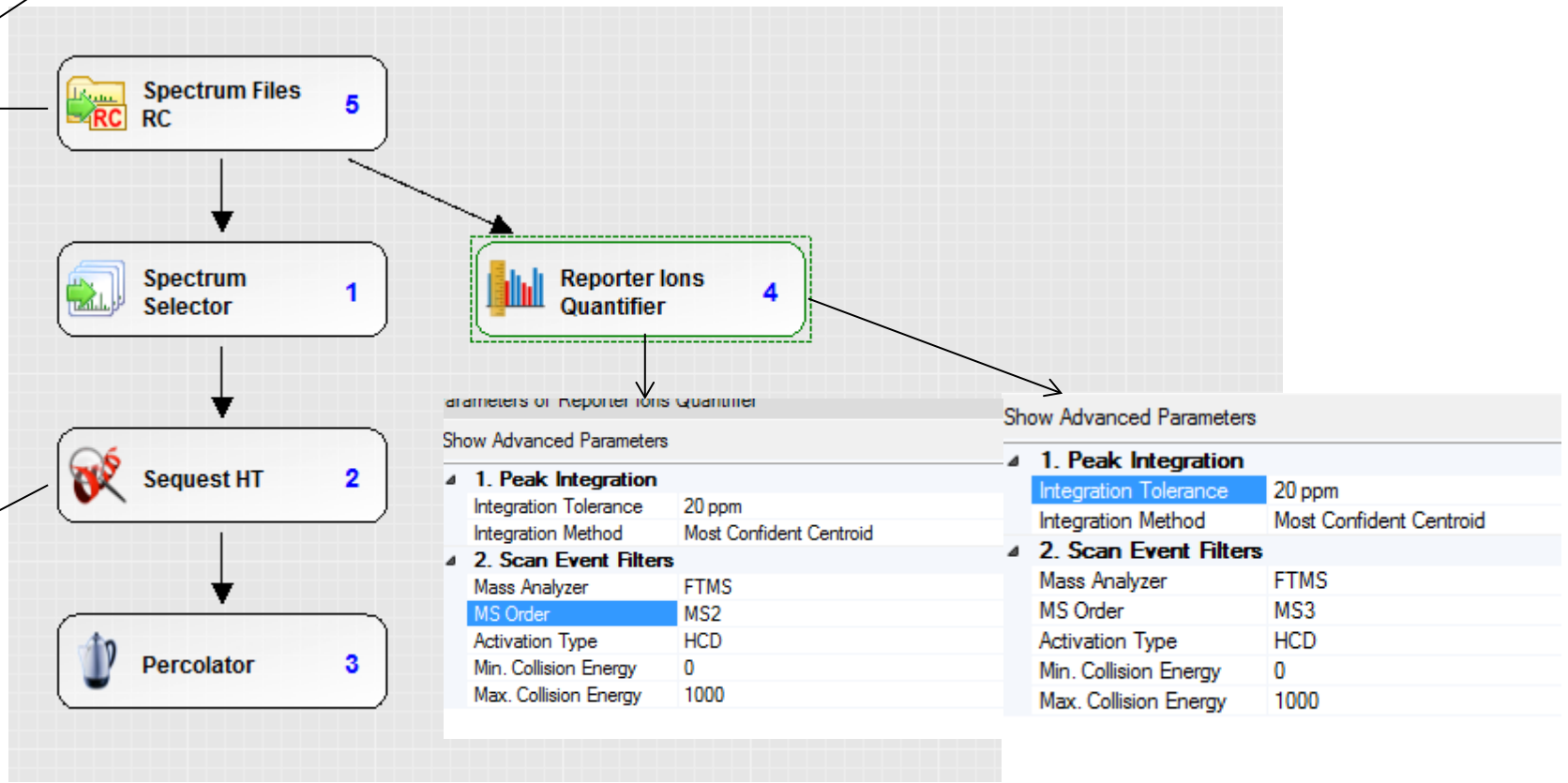


III. Search Parameters For Processing Workflow

Download SwissProt yeast database, taxonomy ID 4932

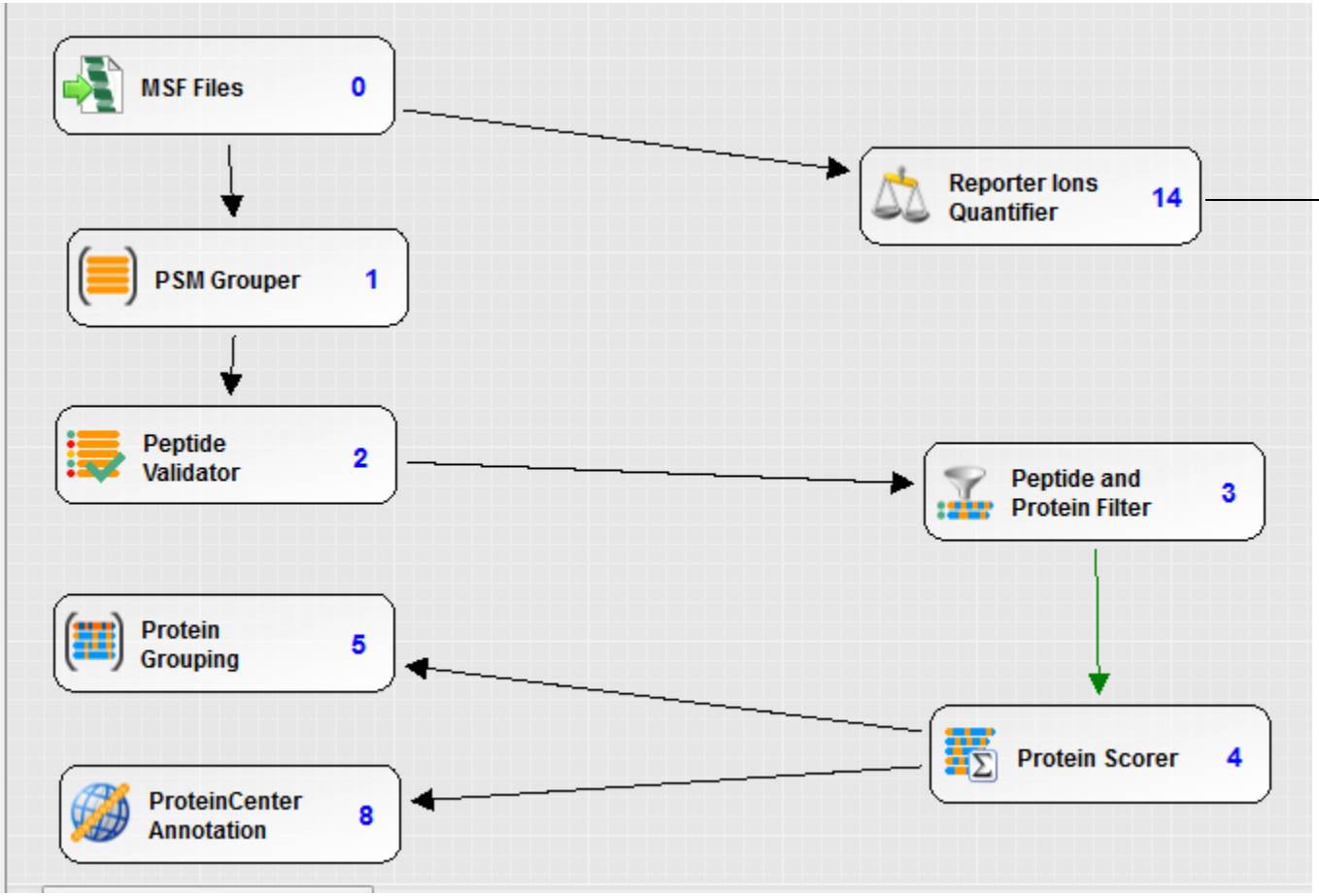






Use 0.02 Da tolerance for MS2 methods and 1.2 Da for SPS methods

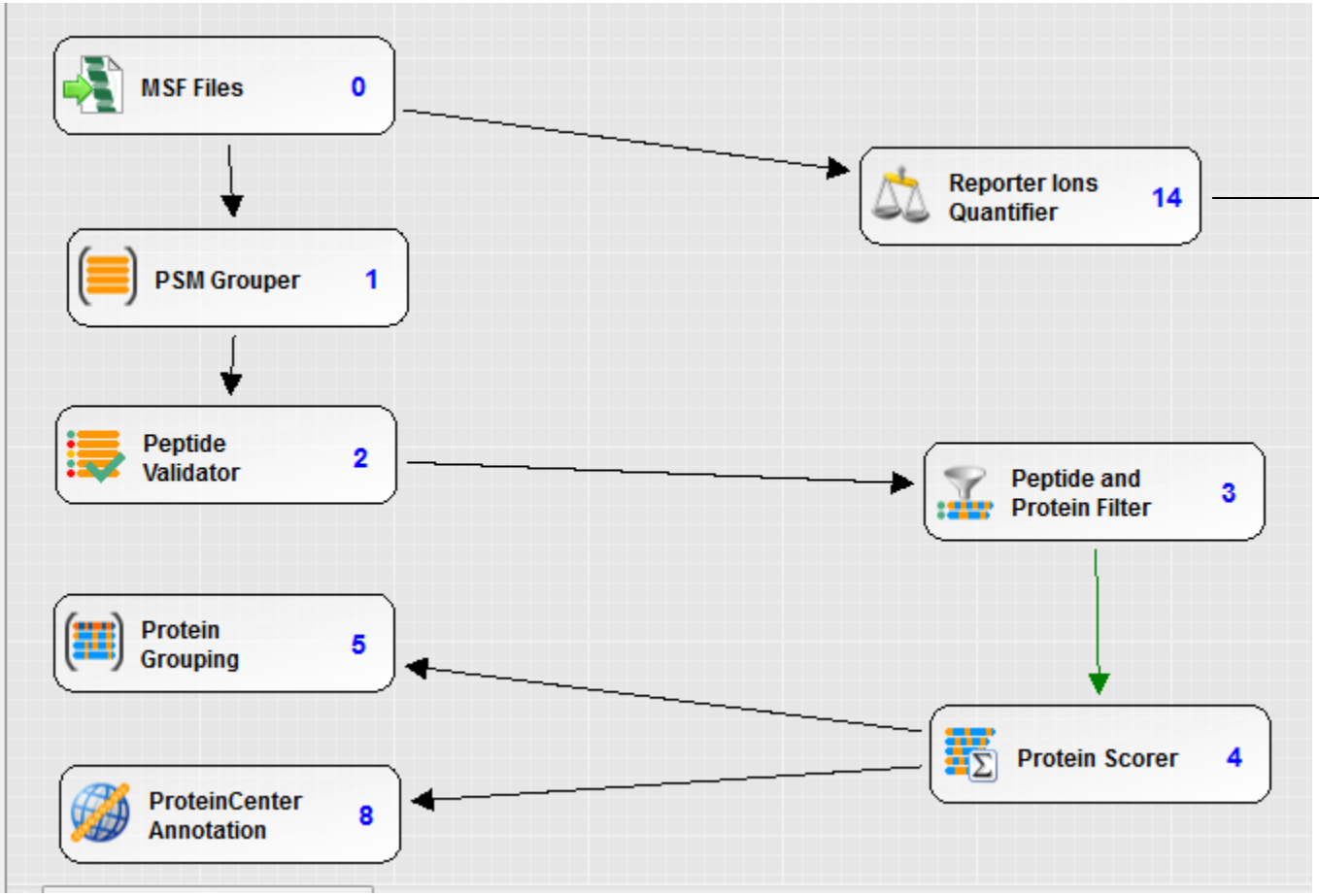
III. Consensus Workflow



1. General Quantification Settings	
Peptides to Use	Unique
Consider Protein Grou	True
Reject Quan Results \	False
2. Reporter Quantification	
Reporter Abundance	Automatic
Apply Quan Value Cor	False
Co-Isolation Threshold	50
Average Reporter S/N	10
3. Normalization and Scaling	
Normalization Mode	None
Proteins For Normaliza	
Scaling Mode	On Controls Average
4. Exclude Peptides from Protein Quantification	
1. Excluded Peptide M	None
2. Excluded Peptide M	None
3. Excluded Peptide M	None
N-Terminal Excluded I	None
5. Quan Rollup _Hypothesis Testing	
Ratio Calculation	Summed Abundance Based
Maximum Allowed Fold	100
Imputation Mode	None
Hypothesis Test	ANOVA (Individual Proteins)
6. Quan Ratio Distributions	
1st Fold Change Thre:	2
2nd Fold Change Thre	4
3rd Fold Change Thre	6

75 for MS3

III. Consensus Workflow: New In PD 2.3 SPS Mass Matches



1. General Quantification Settings	
Peptides to Use	Unique
Consider Protein Grc	True
Reject Quan Results	False
2. Reporter Quantification	
Reporter Abundance	Automatic
Apply Quan Value C	True
Co-Isolation Thresho	75
Average Reporter S.	10
SPS Mass Matches	65
3. Normalization and Scaling	
Normalization Mode	None
Proteins For Normali	
Scaling Mode	On Controls Average
4. Exclude Peptides from Protein Quantification	
For Normalization	Use All Peptides
For Protein Roll-Up	Use All Peptides
For Pairwise Ratios	Exclude Modified
1. Considered Peptic	None
2. Considered Peptic	None
3. Considered Peptic	None
N-Terminal Consider	None
5. Quan Rollup and Hypothesis Testing	
Protein Ratio Calcula	Protein Abundance Based
Maximum Allowed Fc	100
Imputation Mode	None
Hypothesis Test	ANOVA (Individual Proteins)
6. Quan Ratio Distributions	
1st Fold Change Thr	2
2nd Fold Change Th	4

SPS Mass Matches

Sequence: DGQITIITPK, K9-TMT6plex (229.16293 Da), D1-TMT6plex (229.16293 Da)
Charge: +2, Monoisotopic m/z: 715.93493 Da (+0.34 mmu/+0.48 ppm), MH+: 1430.86259 Da, RT: 72.4247 min,
Identified with: Sequest HT (v1.17); XCorr:3.08, Percolator q-Value:1.2e-4, Percolator PEP:6.6e-4,
Fragment match tolerance used for search: 0.6 Da

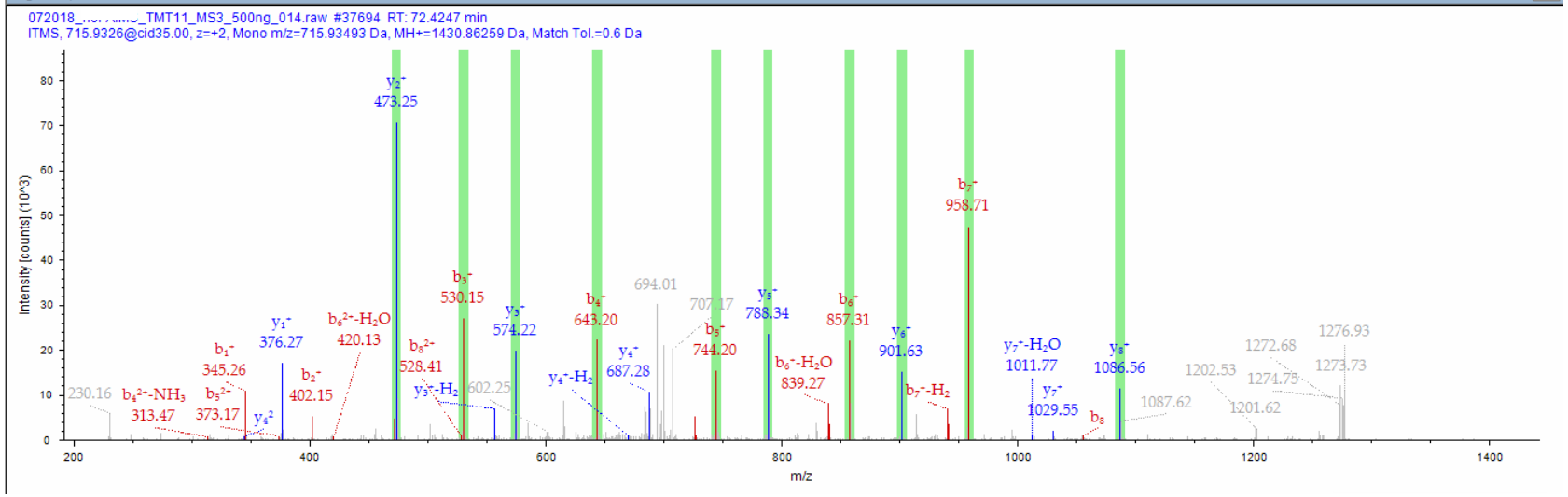
Fragment Matches

Value Type: Theo. Mass [Da]

Ion Series | Neutral Losses | Precursor Ions | Internal Fragments

#1	b+	b2+	Seq.	y+	y2+	#2
1	345.19715	173.10221	D-TMT6plex			9
2	402.21862	201.61295	G	1086.67203	543.83965	8
3	530.27719	265.64223	Q	1029.65056	515.32892	7
4	643.36126	322.18427	I	901.59198	451.29963	6
5	744.40894	372.70811	T	788.50792	394.75760	5
6	857.49300	429.25014	I	687.46024	344.23376	4
7	958.54068	479.77398	T	574.37618	287.69173	3
8	1055.59344	528.30036	P	473.32850	237.16789	2
9			K-TMT6plex	376.27574	188.64151	1

Fragment Spectrum



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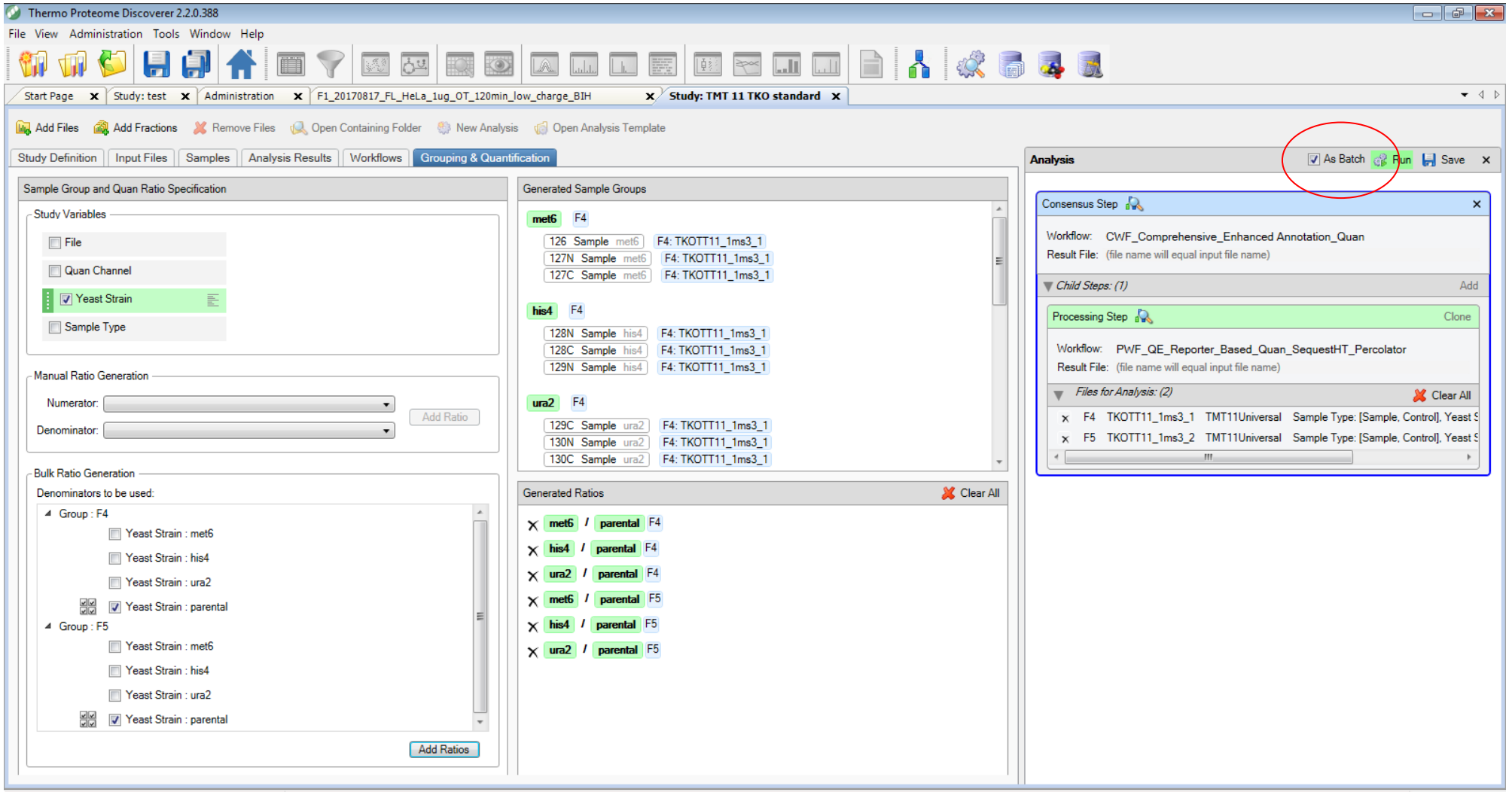
III. Ratios Set up per Individual File



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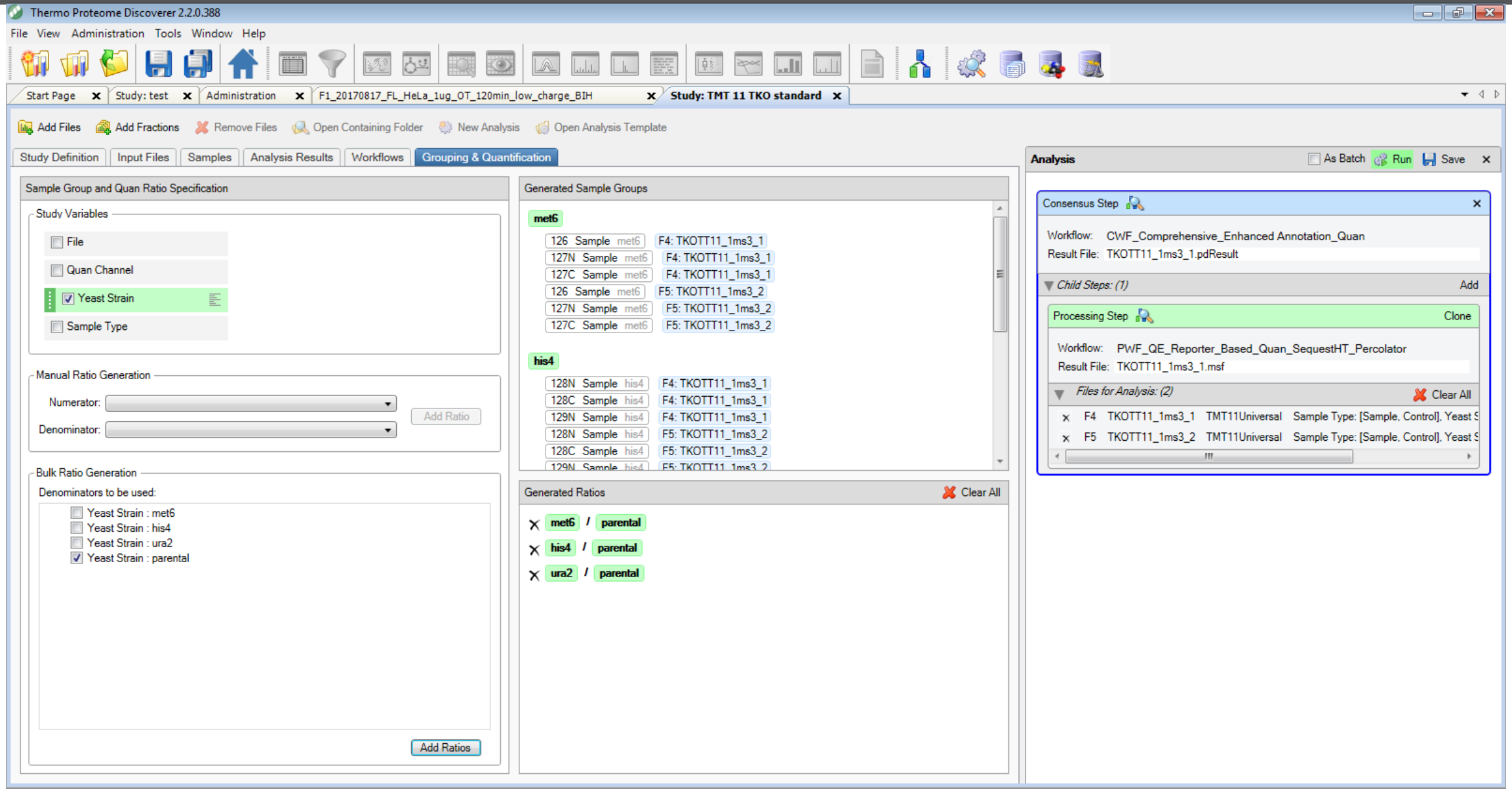
III. Ratios Set up for Multiple files



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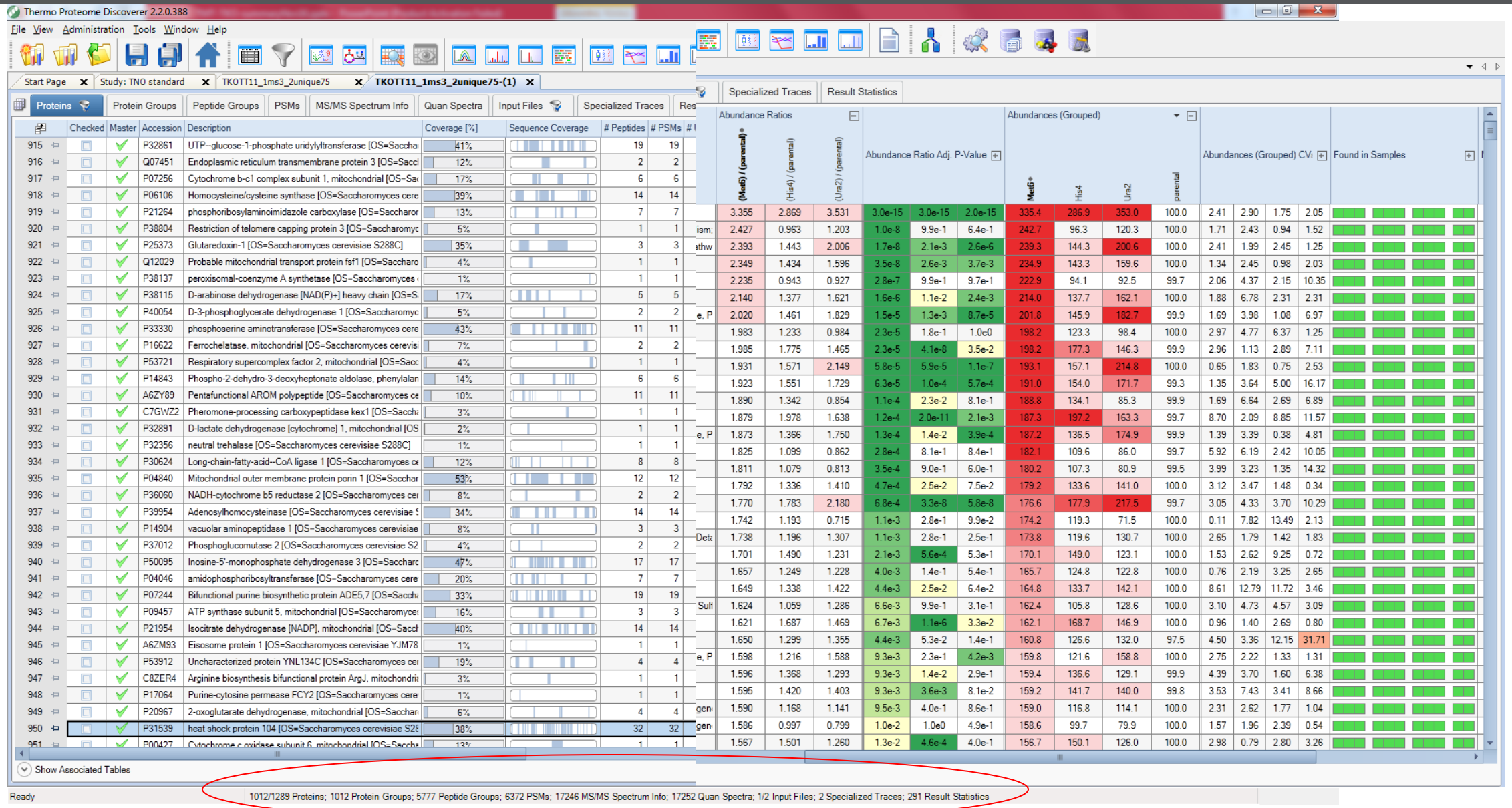
Results and Quality Control of LC-MS System

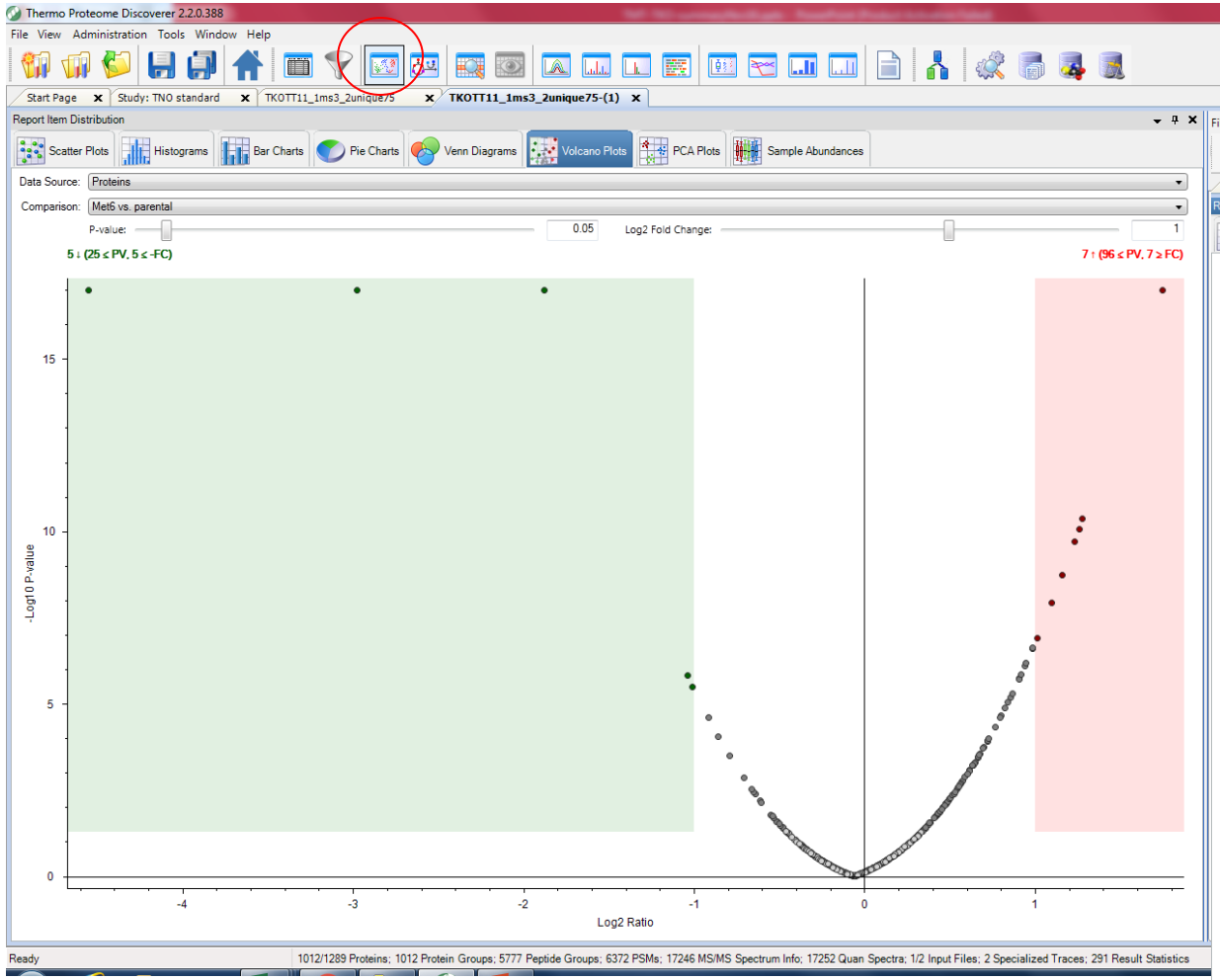
Search and Quan Results: 1012 Protein Groups, 5777 Unique Peptides, 6372 PSMs

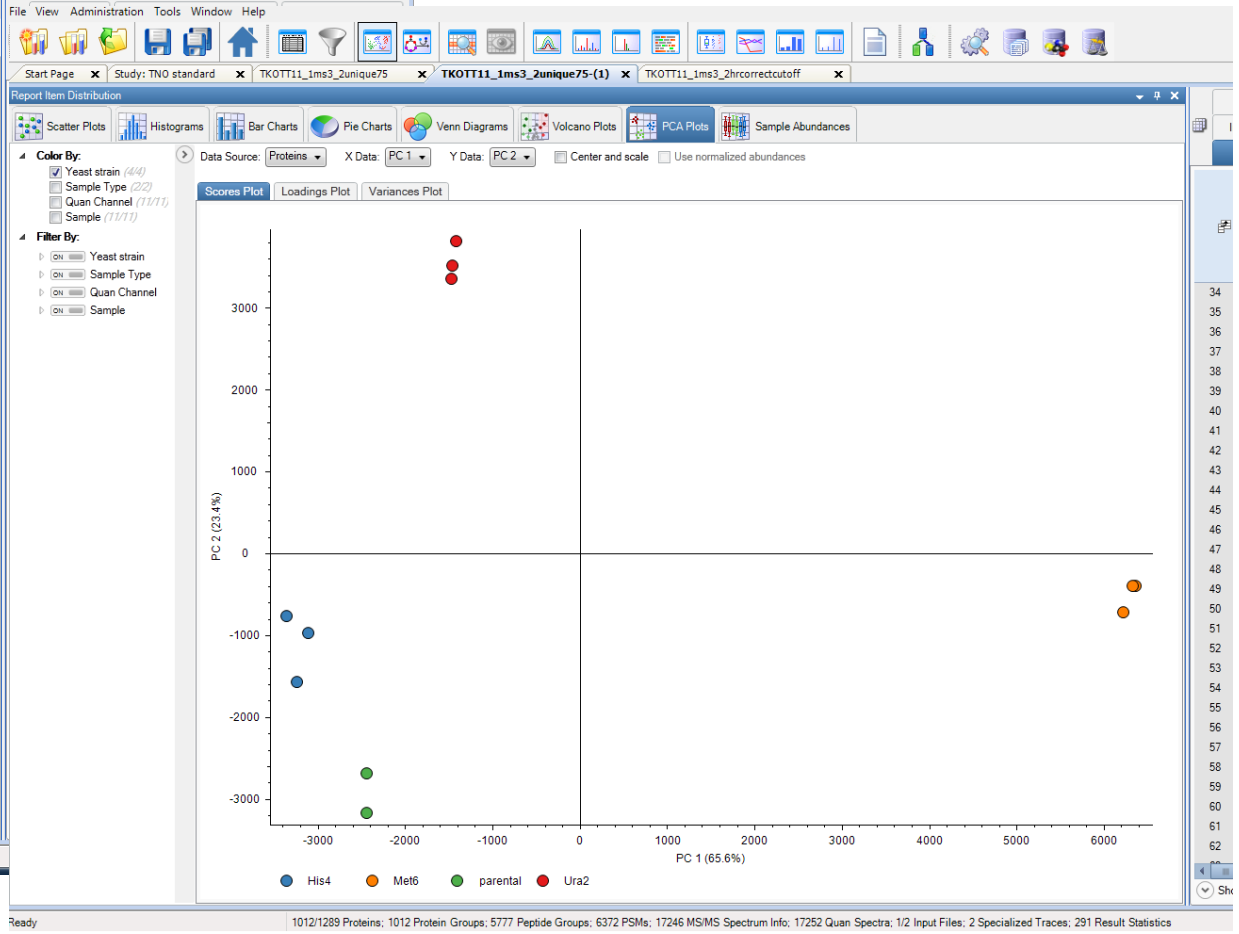


	Checked	Master	Accession	Description	Coverage [%]	Sequence Coverage	# Peptides	# PSMs
915		✓	P32861	UTP--glucose-1-phosphate uridylyltransferase [OS=Saccha	41%		19	19
916		✓	Q07451	Endoplasmic reticulum transmembrane protein 3 [OS=Sacc	12%		2	2
917		✓	P07256	Cytochrome b-c1 complex subunit 1, mitochondrial [OS=Sa	17%		6	6
918		✓	P06106	Homocysteine/cysteine synthase [OS=Saccharomyces cere	39%		14	14
919		✓	P21264	phosphoribosylaminoimidazole carboxylase [OS=Saccharor	13%		7	7
920		✓	P38804	Restriction of telomere capping protein 3 [OS=Saccharomyc	5%		1	1
921		✓	P25373	Glutaredoxin-1 [OS=Saccharomyces cerevisiae S288C]	35%		3	3
922		✓	Q12029	Probable mitochondrial transport protein fsf1 [OS=Saccharo	4%		1	1
923		✓	P38137	peroxisomal-coenzyme A synthetase [OS=Saccharomyces	1%		1	1
924		✓	P38115	D-arabinose dehydrogenase [NAD(P)+] heavy chain [OS=S	17%		5	5
925		✓	P40054	D-3-phosphoglycerate dehydrogenase 1 [OS=Saccharomyc	5%		2	2
926		✓	P33330	phosphoserine aminotransferase [OS=Saccharomyces cere	43%		11	11
927		✓	P16622	Ferrochelatase, mitochondrial [OS=Saccharomyces cerevis	7%		2	2
928		✓	P53721	Respiratory supercomplex factor 2, mitochondrial [OS=Sacc	4%		1	1
929		✓	P14843	Phospho-2-dehydro-3-deoxyheptonate aldolase, phenylalan	14%		6	6
930		✓	A6ZY89	Pentafunctional AROM polypeptide [OS=Saccharomyces ce	10%		11	11
931		✓	C7GWZ2	Pheromone-processing carboxypeptidase kex1 [OS=Sacchi	3%		1	1
932		✓	P32891	D-lactate dehydrogenase [cytochrome] 1, mitochondrial [OS	2%		1	1
933		✓	P32356	neutral trehalase [OS=Saccharomyces cerevisiae S288C]	1%		1	1
934		✓	P30624	Long-chain-fatty-acid--CoA ligase 1 [OS=Saccharomyces ce	12%		8	8
935		✓	P04840	Mitochondrial outer membrane protein porin 1 [OS=Sacchar	53%		12	12
936		✓	P36060	NADH-cytochrome b5 reductase 2 [OS=Saccharomyces cer	8%		2	2
937		✓	P39954	Adenosylhomocysteinase [OS=Saccharomyces cerevisiae S	34%		14	14
938		✓	P14904	vacuolar aminopeptidase 1 [OS=Saccharomyces cerevisiae	8%		3	3
939		✓	P37012	Phosphoglucomutase 2 [OS=Saccharomyces cerevisiae S2	4%		2	2
940		✓	P50095	Inosine-5'-monophosphate dehydrogenase 3 [OS=Sacchare	47%		17	17
941		✓	P04046	amidophosphoribosyltransferase [OS=Saccharomyces cere	20%		7	7
942		✓	P07244	Bifunctional purine biosynthetic protein ADE5,7 [OS=Sacchi	33%		19	19
943		✓	P09457	ATP synthase subunit 5, mitochondrial [OS=Saccharomyce	16%		3	3
944		✓	P21954	Isocitrate dehydrogenase [NADP], mitochondrial [OS=Saccl	40%		14	14
945		✓	A6ZM93	Eisosome protein 1 [OS=Saccharomyces cerevisiae YJM78	1%		1	1
946		✓	P53912	Uncharacterized protein YNL134C [OS=Saccharomyces cer	19%		4	4
947		✓	C8ZER4	Arginine biosynthesis bifunctional protein ArgJ, mitochondri	3%		1	1
948		✓	P17064	Purine-cytosine permease FCY2 [OS=Saccharomyces cere	1%		1	1
949		✓	P20967	2-oxoglutarate dehydrogenase, mitochondrial [OS=Sacchar	6%		4	4
950		✓	P31539	heat shock protein 104 [OS=Saccharomyces cerevisiae S28	38%		32	32
951		✓	P00427	Cytochrome c oxidase subunit 6, mitochondrial [OS=Sacchar	13%		1	1

Abundance Ratios (MetG) / (parental)	Abundance Ratios (His4) / (parental)	Abundance Ratios (Ura2) / (parental)	Abundance Ratio Adj. P-Value			Abundances (Grouped) MetG	Abundances (Grouped) His4	Abundances (Grouped) Ura2	Abundances (Grouped) parental	Abundances (Grouped) CV				Found in Samples			
3.355	2.869	3.531	3.0e-15	3.0e-15	2.0e-15	335.4	286.9	353.0	100.0	2.41	2.90	1.75	2.05				
2.427	0.963	1.203	1.0e-8	9.9e-1	6.4e-1	242.7	96.3	120.3	100.0	1.71	2.43	0.94	1.52				
2.393	1.443	2.006	1.7e-8	2.1e-3	2.6e-6	239.3	144.3	200.6	100.0	2.41	1.99	2.45	1.25				
2.349	1.434	1.596	3.5e-8	2.6e-3	3.7e-3	234.9	143.3	159.6	100.0	1.34	2.45	0.98	2.03				
2.235	0.943	0.927	2.8e-7	9.9e-1	9.7e-1	222.9	94.1	92.5	99.7	2.06	4.37	2.15	10.35				
2.140	1.377	1.621	1.6e-6	1.1e-2	2.4e-3	214.0	137.7	162.1	100.0	1.88	6.78	2.31	2.31				
2.020	1.461	1.829	1.5e-5	1.3e-3	8.7e-5	201.8	145.9	182.7	99.9	1.69	3.98	1.08	6.97				
1.983	1.233	0.984	2.3e-5	1.8e-1	1.0e0	198.2	123.3	98.4	100.0	2.97	4.77	6.37	1.25				
1.985	1.775	1.465	2.3e-5	4.1e-8	3.5e-2	198.2	177.3	146.3	99.9	2.96	1.13	2.89	7.11				
1.931	1.571	2.149	5.8e-5	5.9e-5	1.1e-7	193.1	157.1	214.8	100.0	0.65	1.83	0.75	2.53				
1.923	1.551	1.729	6.3e-5	1.0e-4	5.7e-4	191.0	154.0	171.7	99.3	1.35	3.64	5.00	16.17				
1.890	1.342	0.854	1.1e-4	2.3e-2	8.1e-1	188.8	134.1	85.3	99.9	1.69	6.64	2.69	6.89				
1.879	1.978	1.638	1.2e-4	2.0e-11	2.1e-3	187.3	197.2	163.3	99.7	8.70	2.09	8.85	11.57				
1.873	1.366	1.750	1.3e-4	1.4e-2	3.9e-4	187.2	136.5	174.9	99.9	1.39	3.39	0.38	4.81				
1.825	1.099	0.862	2.8e-4	8.1e-1	8.4e-1	182.1	109.6	86.0	99.7	5.92	6.19	2.42	10.05				
1.811	1.079	0.813	3.5e-4	9.0e-1	6.0e-1	180.2	107.3	80.9	99.5	3.99	3.23	1.35	14.32				
1.792	1.336	1.410	4.7e-4	2.5e-2	7.5e-2	179.2	133.6	141.0	100.0	3.12	3.47	1.48	0.34				
1.770	1.783	2.180	6.8e-4	3.3e-8	5.8e-8	176.6	177.9	217.5	99.7	3.05	4.33	3.70	10.29				
1.742	1.193	0.715	1.1e-3	2.8e-1	9.9e-2	174.2	119.3	71.5	100.0	0.11	7.82	13.49	2.13				
1.738	1.196	1.307	1.1e-3	2.8e-1	2.5e-1	173.8	119.6	130.7	100.0	2.65	1.79	1.42	1.83				
1.701	1.490	1.231	2.1e-3	5.6e-4	5.3e-1	170.1	149.0	123.1	100.0	1.53	2.62	9.25	0.72				
1.657	1.249	1.228	4.0e-3	1.4e-1	5.4e-1	165.7	124.8	122.8	100.0	0.76	2.19	3.25	2.65				
1.649	1.338	1.422	4.4e-3	2.5e-2	6.4e-2	164.8	133.7	142.1	100.0	8.61	12.79	11.72	3.46				
1.624	1.059	1.286	6.6e-3	9.9e-1	3.1e-1	162.4	105.8	128.6	100.0	3.10	4.73	4.57	3.09				
1.621	1.687	1.469	6.7e-3	1.1e-6	3.3e-2	162.1	168.7	146.9	100.0	0.96	1.40	2.69	0.80				
1.650	1.299	1.355	4.4e-3	5.3e-2	1.4e-1	160.8	126.6	132.0	97.5	4.50	3.36	12.15	31.71				
1.598	1.216	1.588	9.3e-3	2.3e-1	4.2e-3	159.8	121.6	158.8	100.0	2.75	2.22	1.33	1.31				
1.596	1.368	1.293	9.3e-3	1.4e-2	2.9e-1	159.4	136.6	129.1	99.9	4.39	3.70	1.60	6.38				
1.595	1.420	1.403	9.3e-3	3.6e-3	8.1e-2	159.2	141.7	140.0	99.8	3.53	7.43	3.41	8.66				
1.590	1.168	1.141	9.5e-3	4.0e-1	8.6e-1	159.0	116.8	114.1	100.0	2.31	2.62	1.77	1.04				
1.586	0.997	0.799	1.0e-2	1.0e0	4.9e-1	158.6	99.7	79.9	100.0	1.57	1.96	2.39	0.54				
1.567	1.501	1.260	1.3e-2	4.6e-4	4.0e-1	156.7	150.1	126.0	100.0	2.98	0.79	2.80	3.26				

Result Statistics





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Pathway Analysis: Δmet6 & Δura2

Δura2 Over-represented Pathways:

- Reactome
  - Pyrimidine biosynthesis
  - Metabolism of amino acids and derivatives
  - Nucleobase biosynthesis
- KEGG
  - Biosynthesis of antibiotics
  - Alanine, aspartate and glutamate metabolism
  - Pyrimidine metabolism
  - Glycine, serine and threonine metabolism
  - Biosynthesis of secondary metabolites
  - One carbon pool by folate
  - Glyoxylate and dicarboxylate metabolism
- BioCyc
  - UMP biosynthesis II
  - urea cycle
  - Nitrogen Compounds Metabolism
  - Pyrimidine Ribonucleotides De Novo Biosynthesis
  - de novo biosynthesis of pyrimidine ribonucleotides

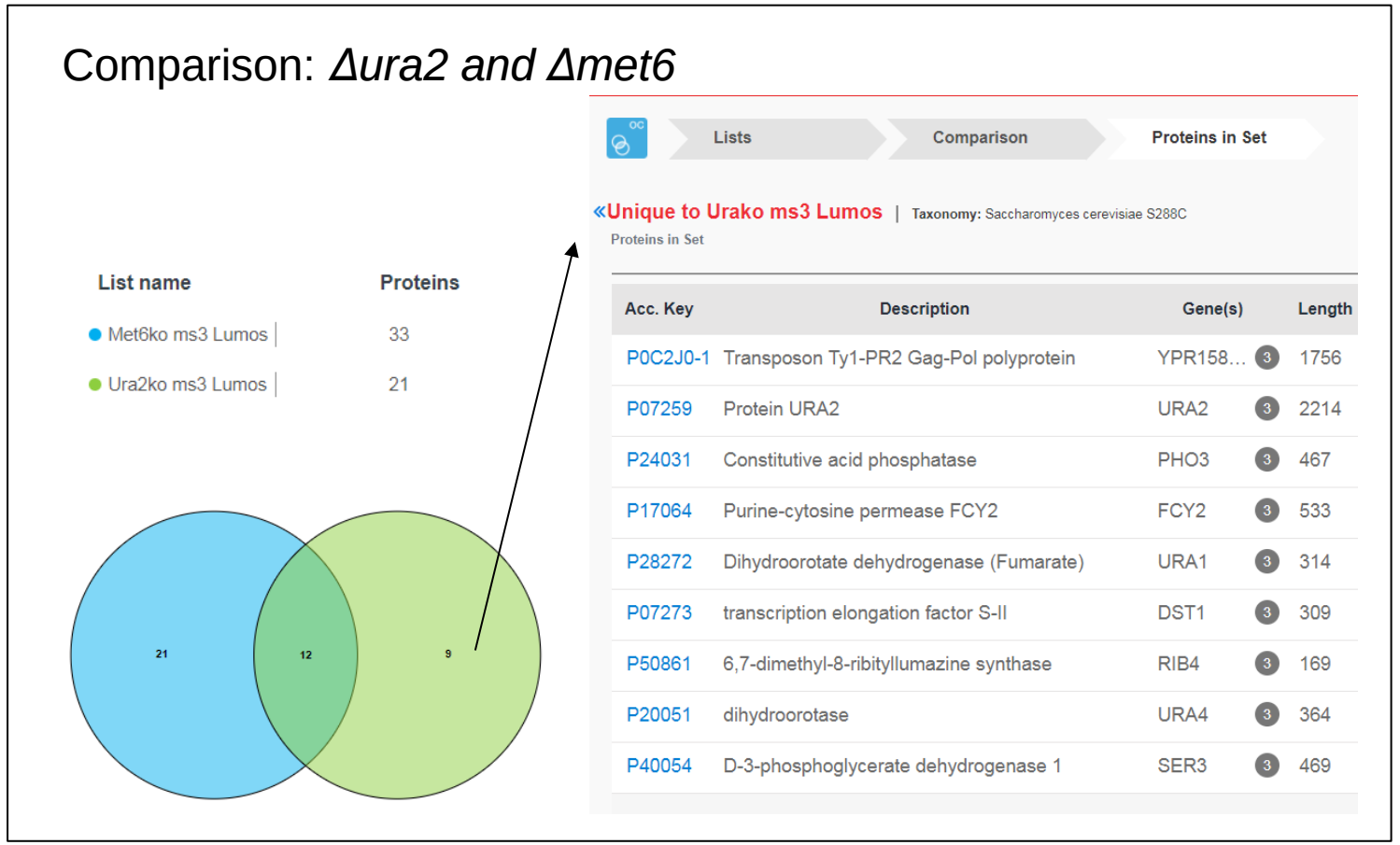
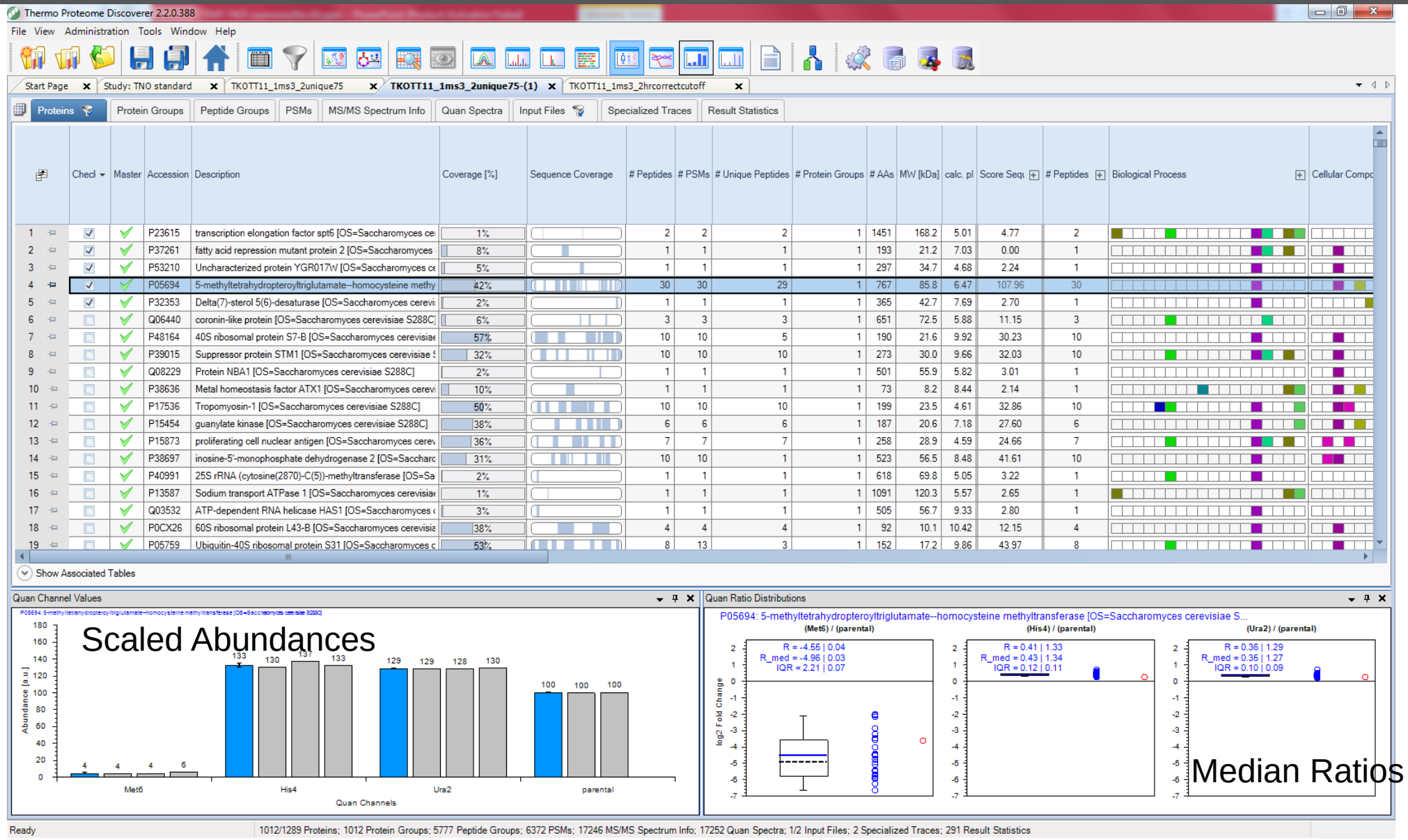


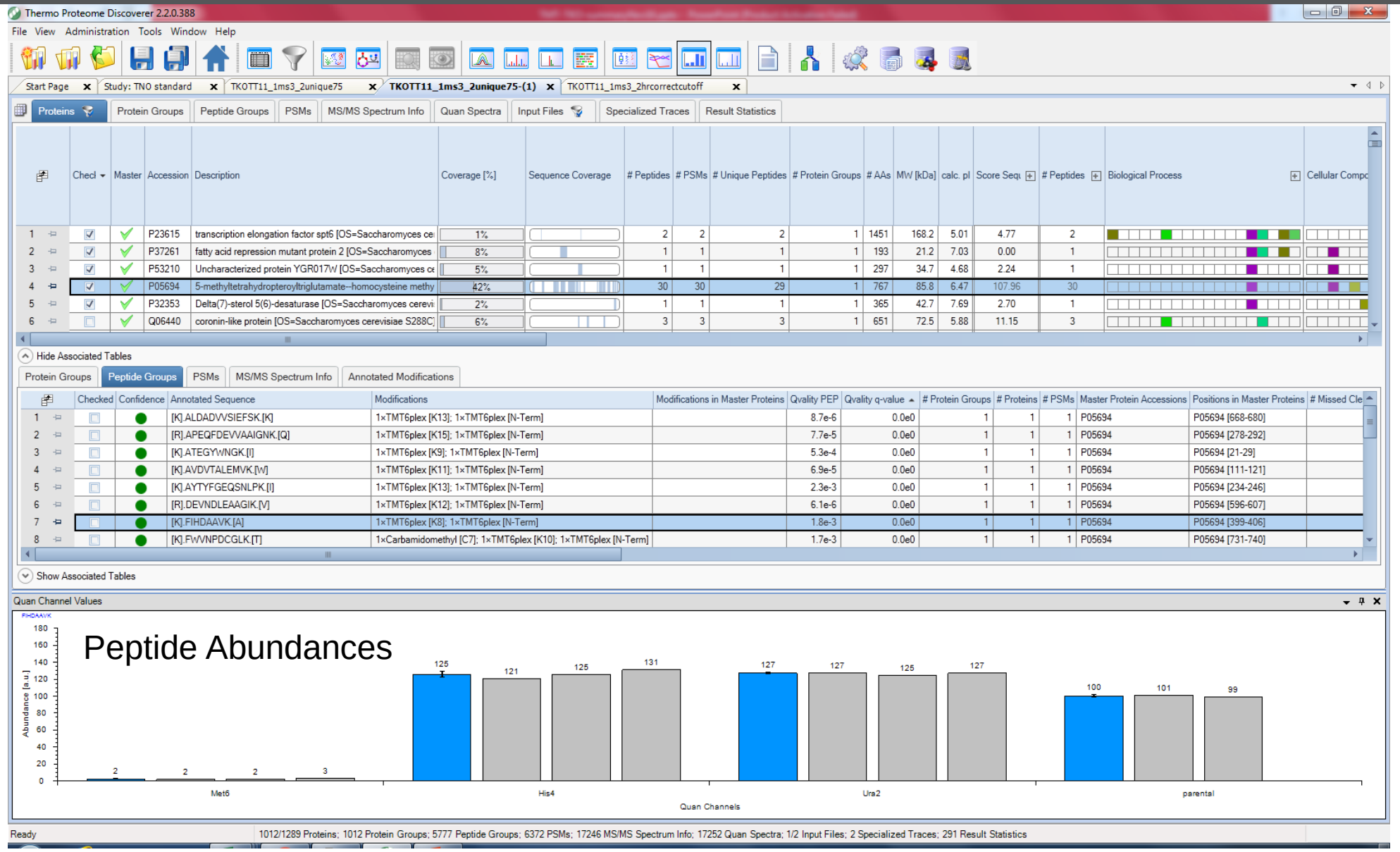
Diagram created with Omics Comparator https://apps.thermofisher.com/apps/lsms/oc

Detailed Result for Selected Protein- KO Protein Met6



	Checl	Master	Accession	Description	Coverage [%]	Sequence Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Seq	# Peptides	Biological Process	Cellular Compc
1	☑	✓	P23615	transcription elongation factor spt6 [OS=Saccharomyces ce	1%		2	2	2	1	1451	168.2	5.01	4.77	2		
2	☑	✓	P37261	fatty acid repression mutant protein 2 [OS=Saccharomyces	8%		1	1	1	1	193	21.2	7.03	0.00	1		
3	☑	✓	P53210	Uncharacterized protein YGR017W [OS=Saccharomyces ce	5%		1	1	1	1	297	34.7	4.68	2.24	1		
4	☑	✓	P05694	5-methyltetrahydropteroyltriglutamate--homocysteine methy	42%		30	30	29	1	767	85.8	6.47	107.96	30		
5	☑	✓	P32353	Delta(7)-sterol 5(6)-desaturase [OS=Saccharomyces cerevi	2%		1	1	1	1	365	42.7	7.69	2.70	1		
6	☐	✓	Q06440	coronin-like protein [OS=Saccharomyces cerevisiae S288C]	6%		3	3	3	1	651	72.5	5.88	11.15	3		
7	☐	✓	P48164	40S ribosomal protein S7-B [OS=Saccharomyces cerevisiae	57%		10	10	5	1	190	21.6	9.92	30.23	10		
8	☐	✓	P39015	Suppressor protein STM1 [OS=Saccharomyces cerevisiae S	32%		10	10	10	1	273	30.0	9.66	32.03	10		
9	☐	✓	Q08229	Protein NBA1 [OS=Saccharomyces cerevisiae S288C]	2%		1	1	1	1	501	55.9	5.82	3.01	1		
10	☐	✓	P38636	Metal homeostasis factor ATX1 [OS=Saccharomyces cerevi	10%		1	1	1	1	73	8.2	8.44	2.14	1		
11	☐	✓	P17536	Tropomyosin-1 [OS=Saccharomyces cerevisiae S288C]	50%		10	10	10	1	199	23.5	4.61	32.86	10		
12	☐	✓	P15454	guanylate kinase [OS=Saccharomyces cerevisiae S288C]	38%		6	6	6	1	187	20.6	7.18	27.60	6		
13	☐	✓	P15873	proliferating cell nuclear antigen [OS=Saccharomyces cerev	36%		7	7	7	1	258	28.9	4.59	24.66	7		
14	☐	✓	P38697	inosine-5'-monophosphate dehydrogenase 2 [OS=Saccharc	31%		10	10	1	1	523	56.5	8.48	41.61	10		
15	☐	✓	P40991	25S rRNA (cytosine(2870)-C(5))-methyltransferase [OS=Sa	2%		1	1	1	1	618	69.8	5.05	3.22	1		
16	☐	✓	P13587	Sodium transport ATPase 1 [OS=Saccharomyces cerevisiae	1%		1	1	1	1	1091	120.3	5.57	2.65	1		
17	☐	✓	Q03532	ATP-dependent RNA helicase HAS1 [OS=Saccharomyces c	3%		1	1	1	1	505	56.7	9.33	2.80	1		
18	☐	✓	P0CX26	60S ribosomal protein L43-B [OS=Saccharomyces cerevisia	38%		4	4	4	1	92	10.1	10.42	12.15	4		
19	☐	✓	P05759	Ubiquitin-40S ribosomal protein S31 [OS=Saccharomyces c	53%		8	13	3	1	152	17.2	9.86	43.97	8		

Detailed Result for Selected Peptide Group from KO Protein Met6

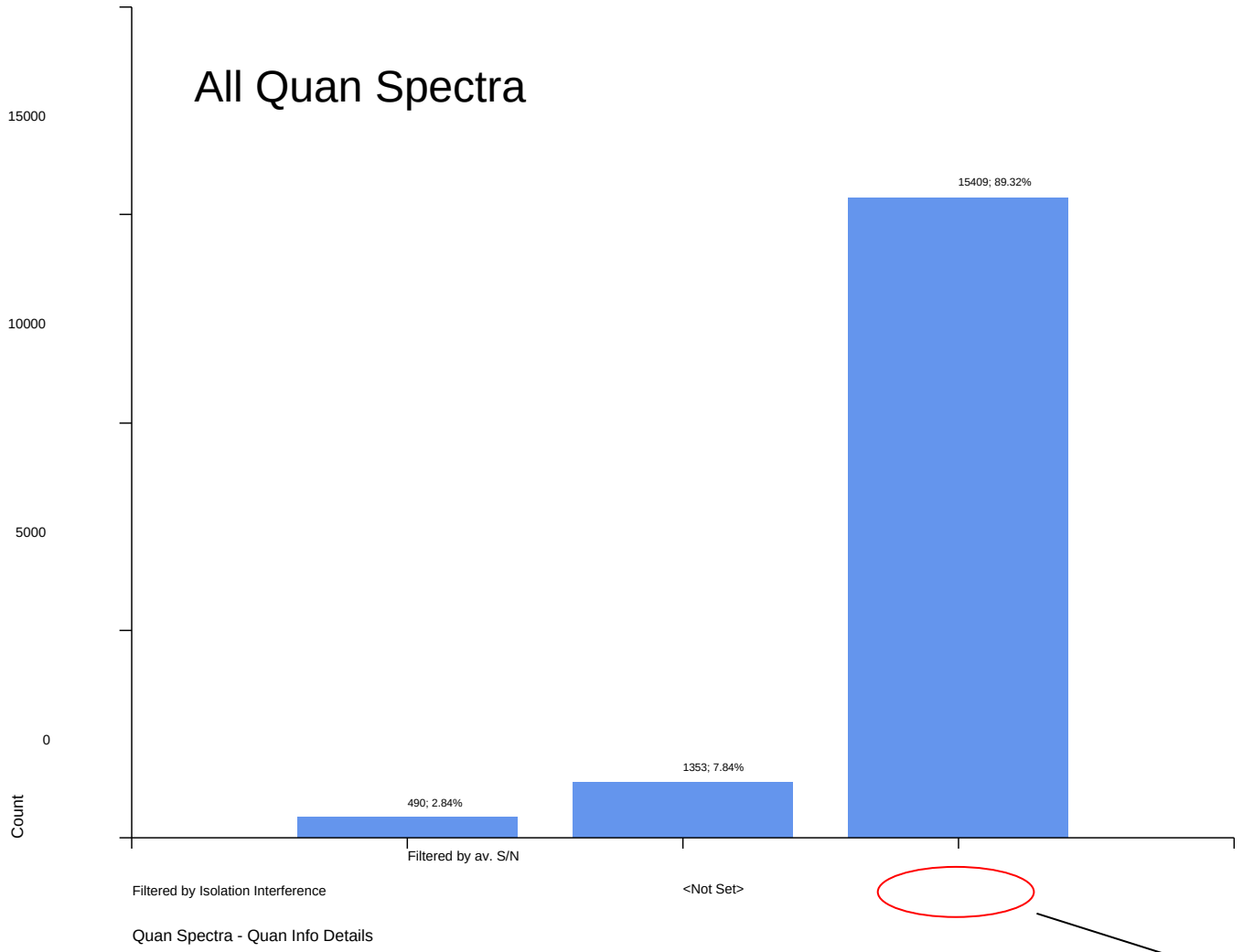


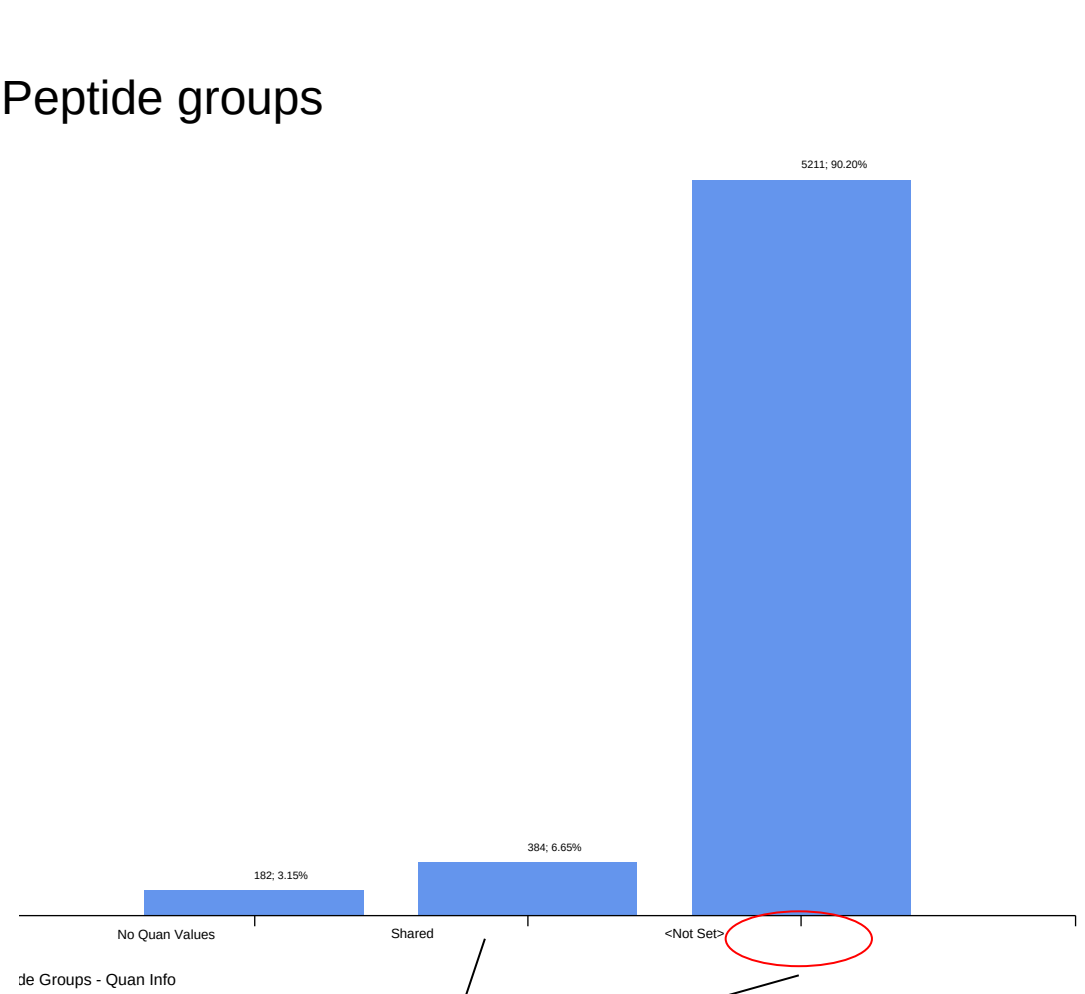
	Checl	Master	Accession	Description	Coverage [%]	Sequence Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Sequ	# Peptides	Biological Process	Cellular Compc
1	☑	✓	P23615	transcription elongation factor spt6 [OS=Saccharomyces ce	1%		2	2	2	1	1451	168.2	5.01	4.77	2		
2	☑	✓	P37261	fatty acid repression mutant protein 2 [OS=Saccharomyces	8%		1	1	1	1	193	21.2	7.03	0.00	1		
3	☑	✓	P53210	Uncharacterized protein YGR017W [OS=Saccharomyces ce	5%		1	1	1	1	297	34.7	4.68	2.24	1		
4	☑	✓	P05694	5-methyltetrahydropteroyltriglutamate--homocysteine methy	42%		30	30	29	1	767	85.8	6.47	107.96	30		
5	☑	✓	P32353	Delta(7)-sterol 5(6)-desaturase [OS=Saccharomyces cerevi	2%		1	1	1	1	365	42.7	7.69	2.70	1		
6	☐	✓	Q06440	coronin-like protein [OS=Saccharomyces cerevisiae S288C]	6%		3	3	3	1	651	72.5	5.88	11.15	3		

Protein Groups | Peptide Groups | PSMs | MS/MS Spectrum Info | Annotated Modifications

	Checked	Confidence	Annotated Sequence	Modifications	Modifications in Master Proteins	Qvality PEP	Qvality q-value	# Protein Groups	# Proteins	# PSMs	Master Protein Accessions	Positions in Master Proteins	# Missed Cle
1	☐	●	[K].ALDADVVSIEFSK.[K]	1×TMT6plex [K13]; 1×TMT6plex [N-Term]		8.7e-6	0.0e0	1	1	1	P05694	P05694 [668-680]	
2	☐	●	[R].APEQFDEVVAAIGNK.[Q]	1×TMT6plex [K15]; 1×TMT6plex [N-Term]		7.7e-5	0.0e0	1	1	1	P05694	P05694 [278-292]	
3	☐	●	[K].ATEGYWNGK.[I]	1×TMT6plex [K9]; 1×TMT6plex [N-Term]		5.3e-4	0.0e0	1	1	1	P05694	P05694 [21-29]	
4	☐	●	[K].AVDVTALEMVK.[W]	1×TMT6plex [K11]; 1×TMT6plex [N-Term]		6.9e-5	0.0e0	1	1	1	P05694	P05694 [111-121]	
5	☐	●	[K].AYTYFGEQSNLPK.[I]	1×TMT6plex [K13]; 1×TMT6plex [N-Term]		2.3e-3	0.0e0	1	1	1	P05694	P05694 [234-246]	
6	☐	●	[R].DEVNDLEAAGIK.[V]	1×TMT6plex [K12]; 1×TMT6plex [N-Term]		6.1e-6	0.0e0	1	1	1	P05694	P05694 [596-607]	
7	☐	●	[K].FIHDAAVK.[A]	1×TMT6plex [K8]; 1×TMT6plex [N-Term]		1.8e-3	0.0e0	1	1	1	P05694	P05694 [399-406]	
8	☐	●	[K].FWVNPDCGLK.[T]	1×Carbamidomethyl [C7]; 1×TMT6plex [K10]; 1×TMT6plex [N-Term]		1.7e-3	0.0e0	1	1	1	P05694	P05694 [731-740]	

Quan Results- Details peptides





Quantified Spectra

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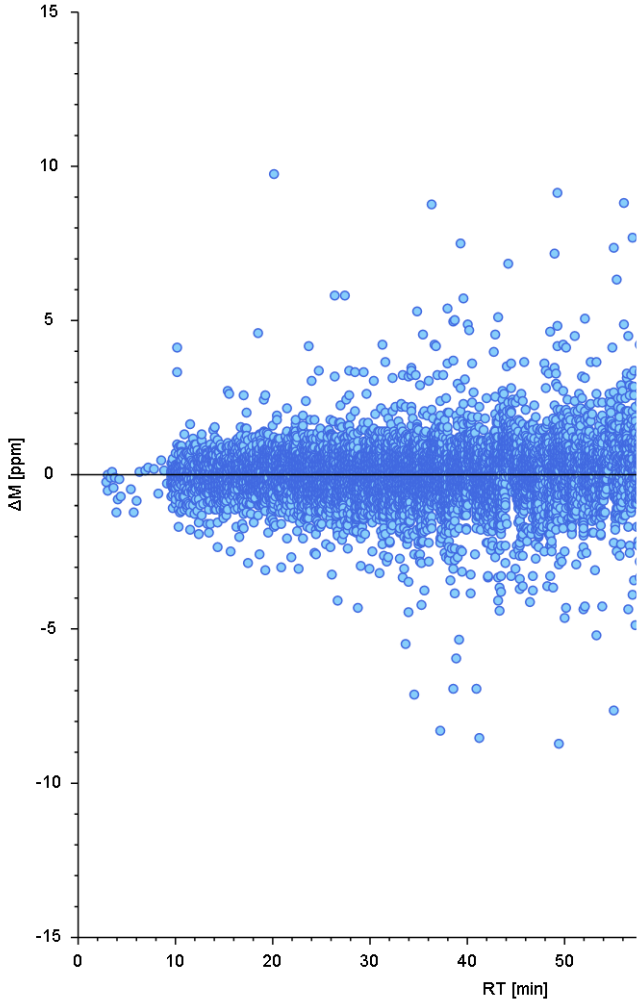
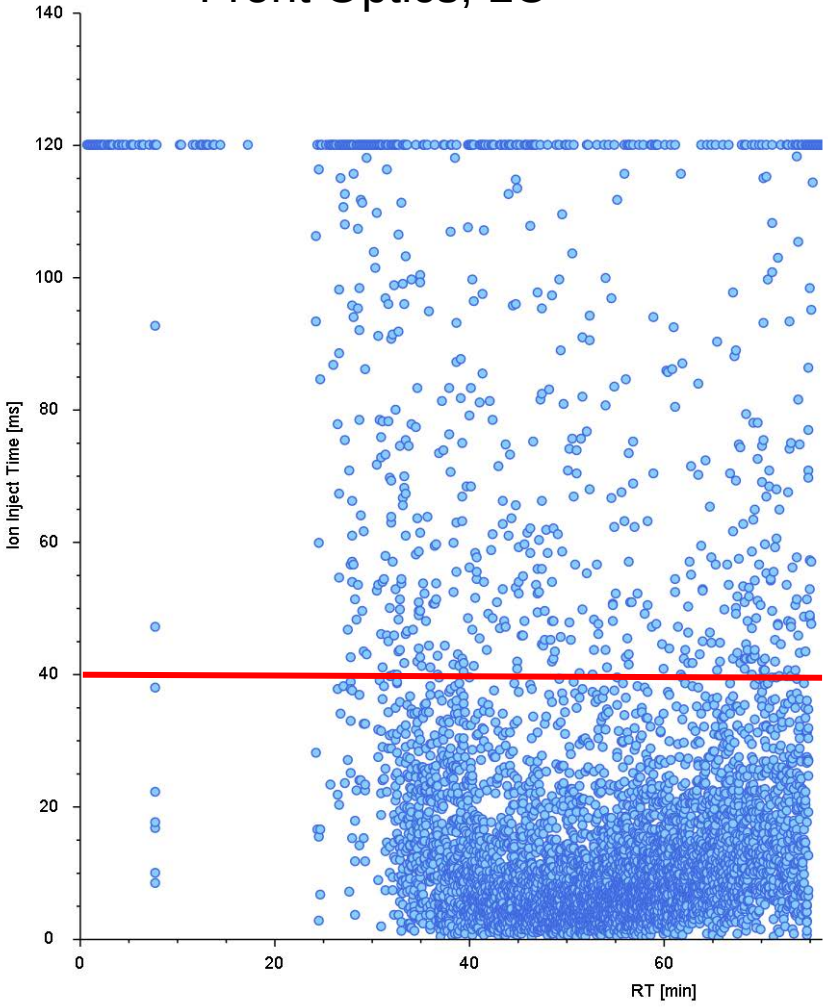
QC Tools- Instrument

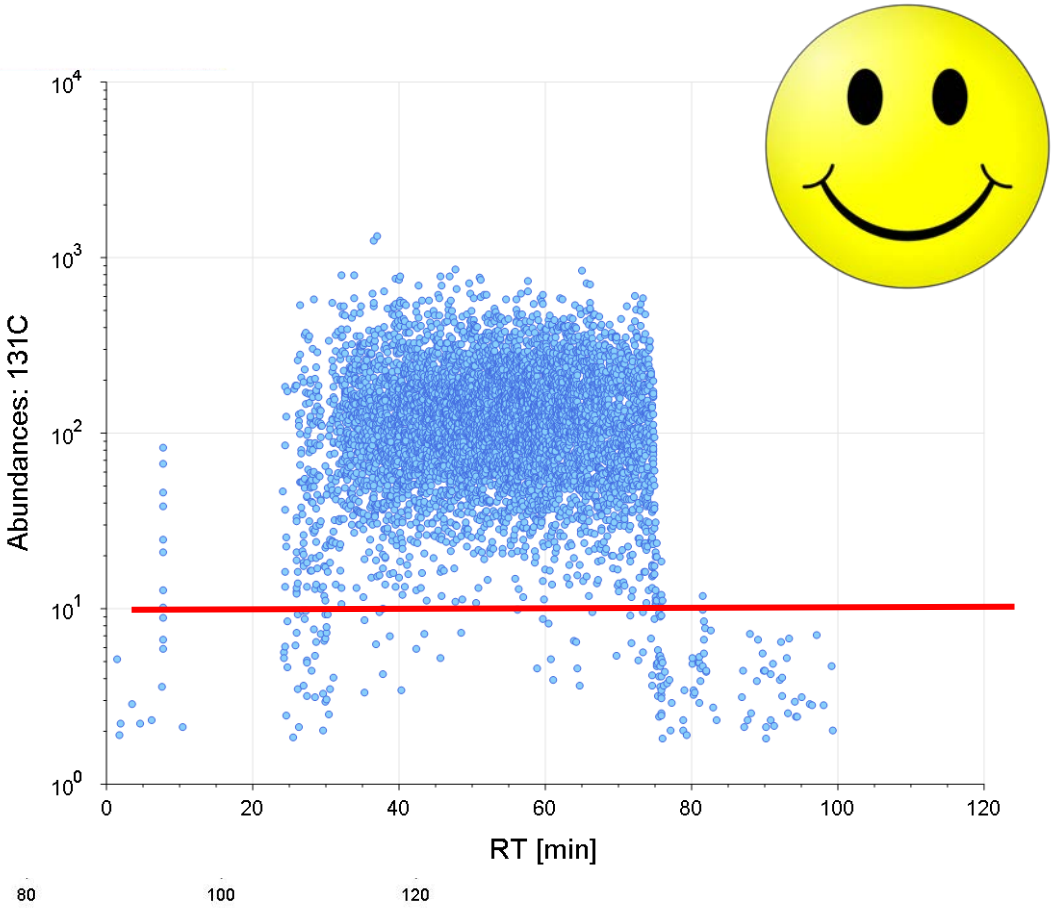
Mass Accuracy



MS2 Injection Time, Front Optics, LC



Reporter Ions S/N; Quad Status



Example Plots for Lumos SPS, 50 min QC run

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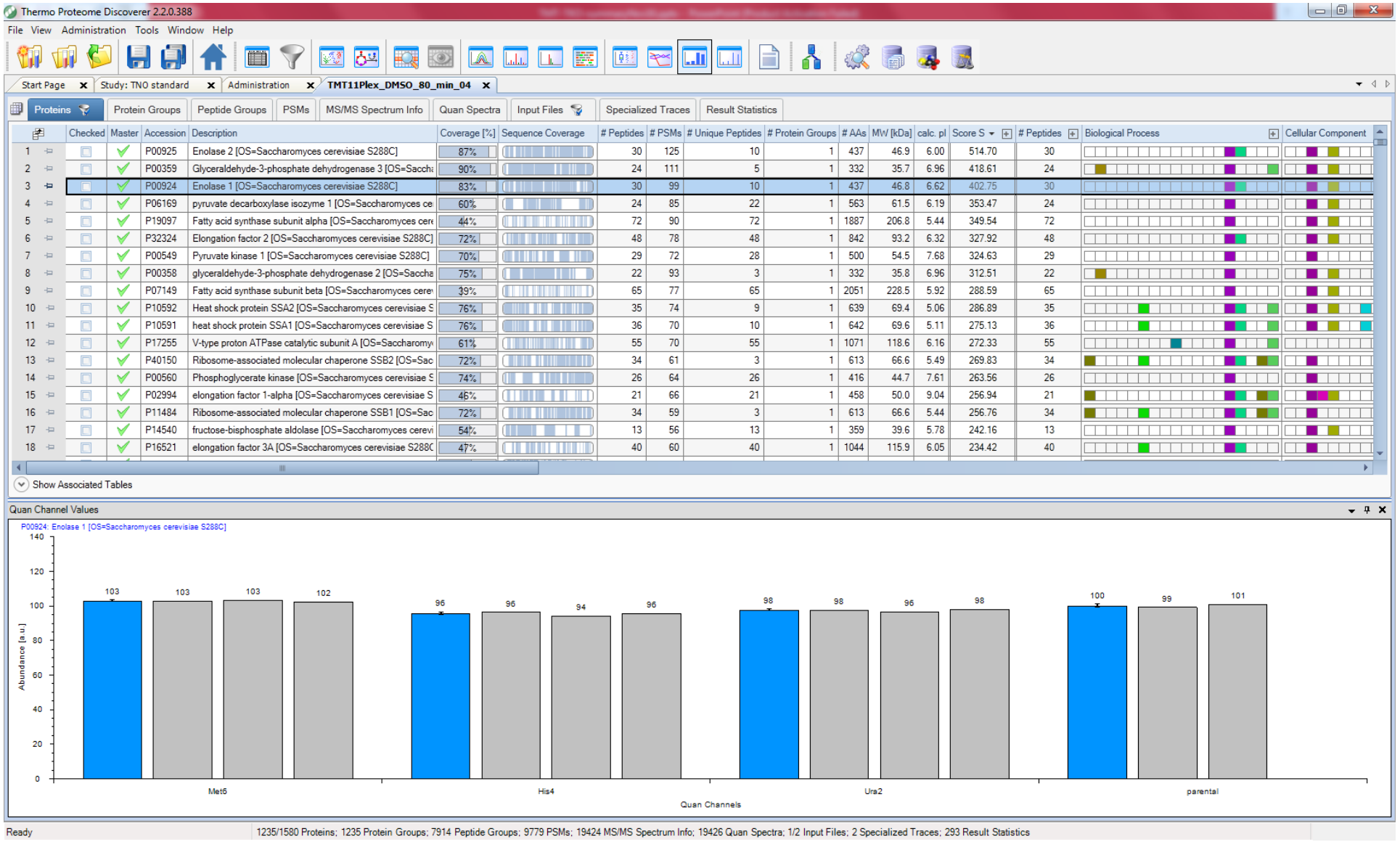
QC Tools Instrument: Housekeeping Proteins Ratios Should be a Perfect 1



- Scaled ,
- No normalization
- Correction factors applied

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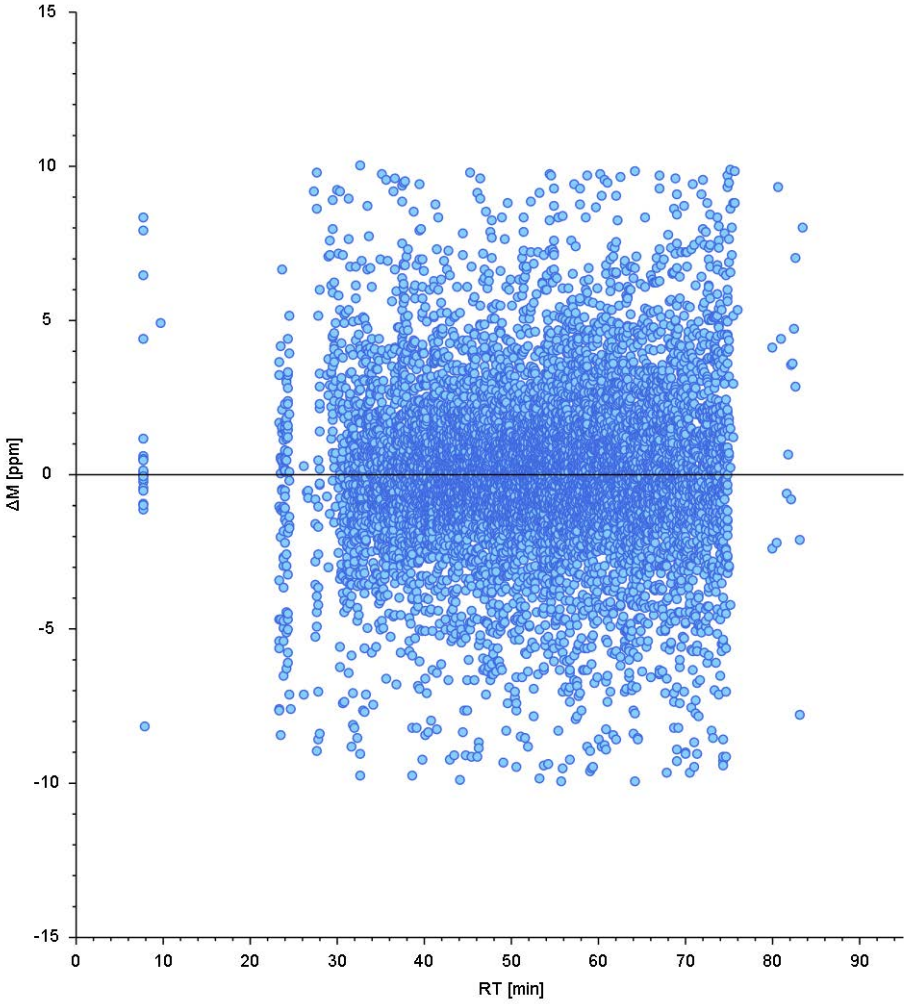
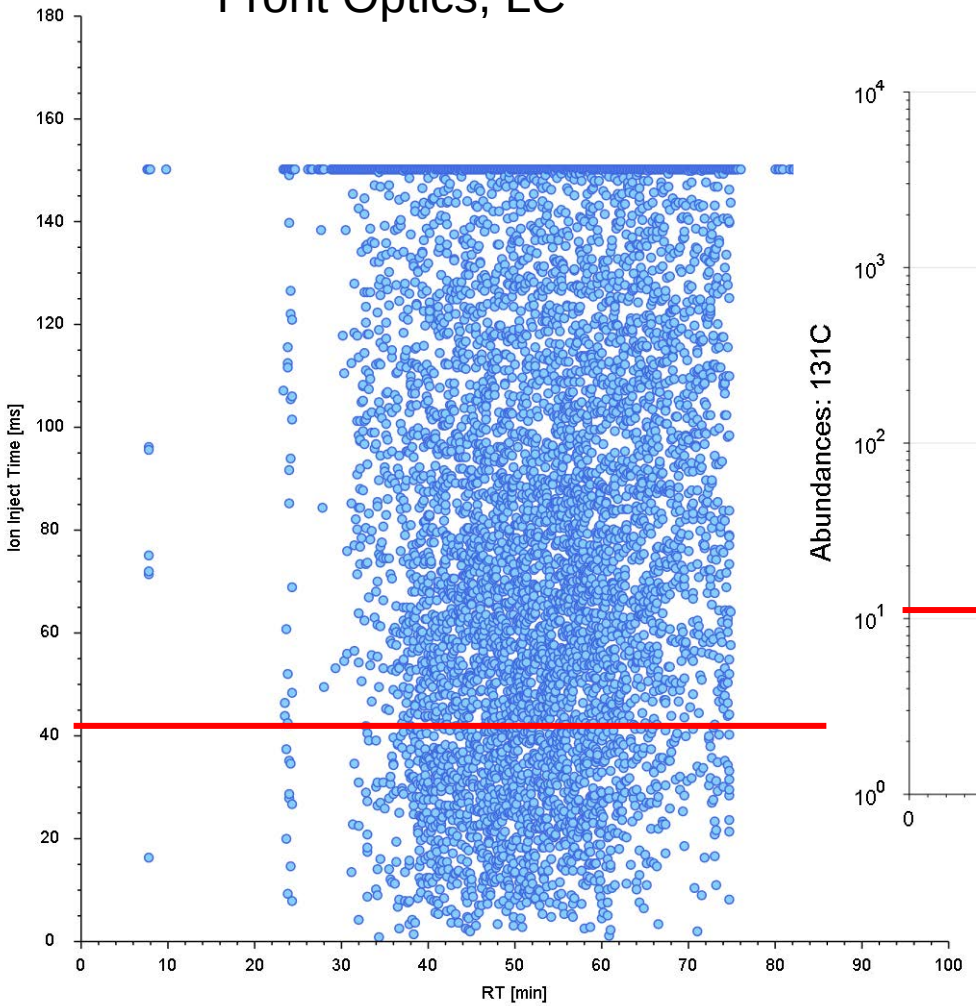
QC Tools- Instrument

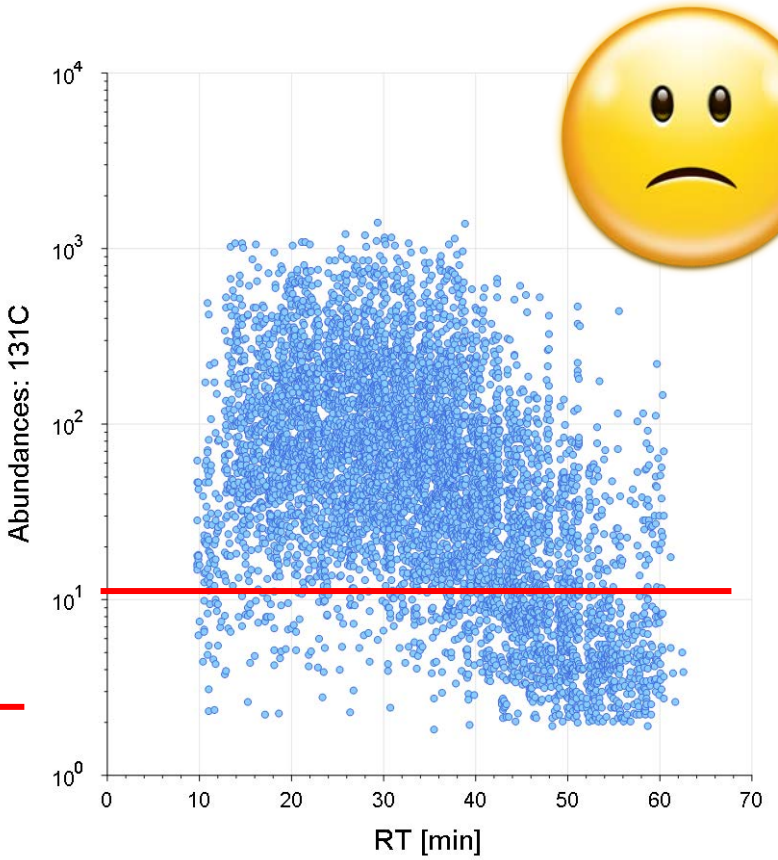
Mass Accuracy



MS2 Injection Time, Front Optics, LC



Reporter Ions S/N; Quad Status



Fusion SPS data, instrument needs maintenance

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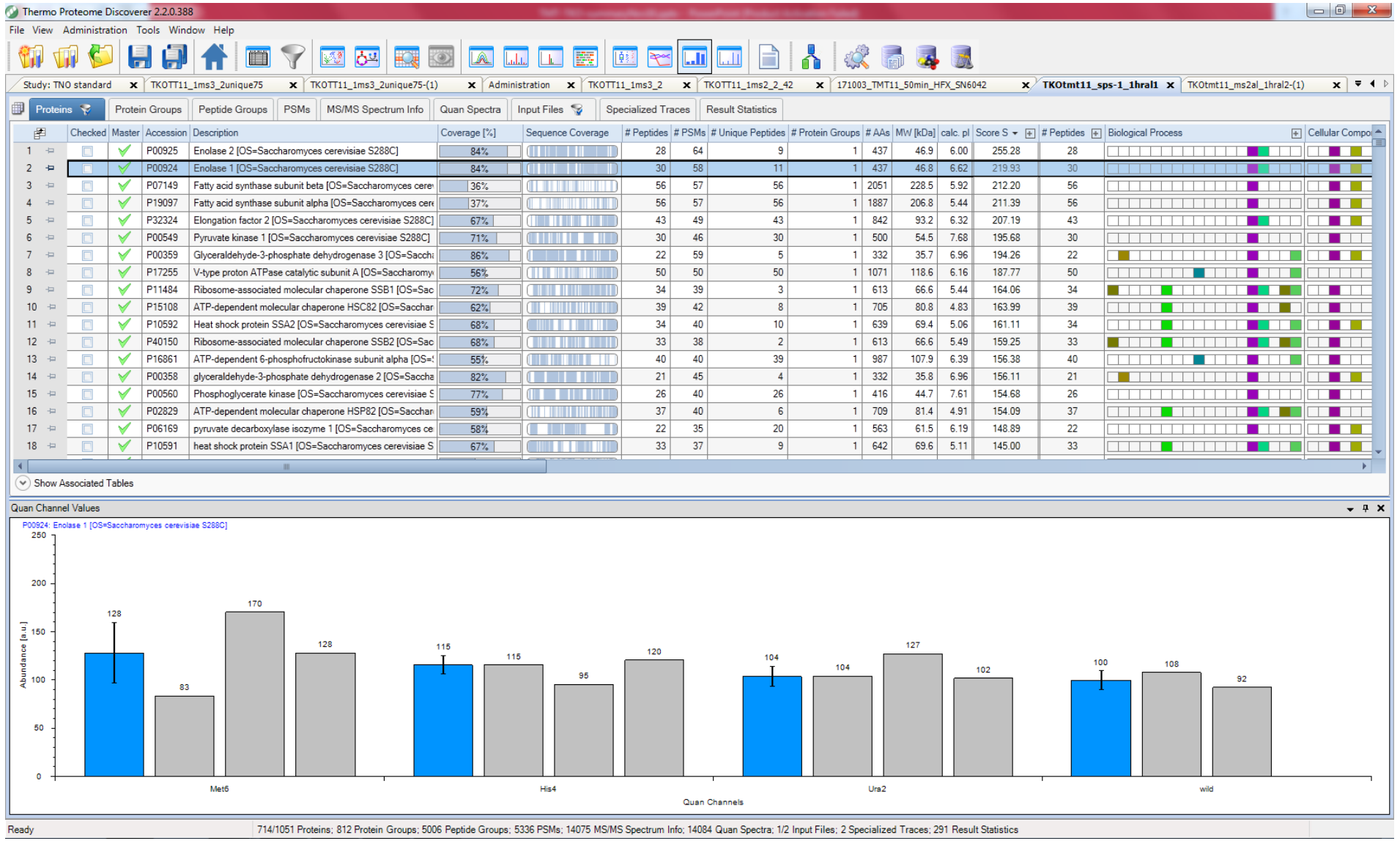
QC Tools Instrument: Housekeeping Proteins Ratios Should be a Perfect 1



- Scaled ,
- No normalization
- Correction factors applied

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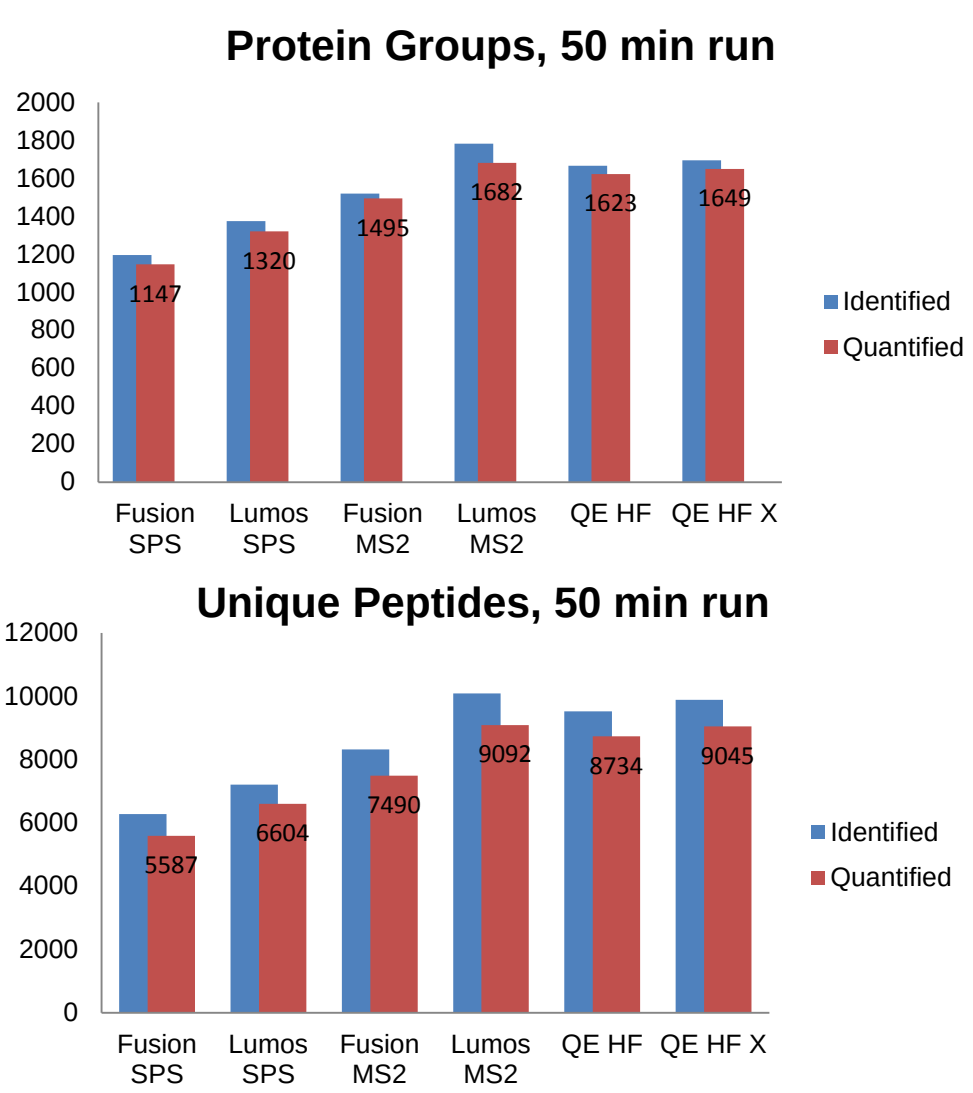
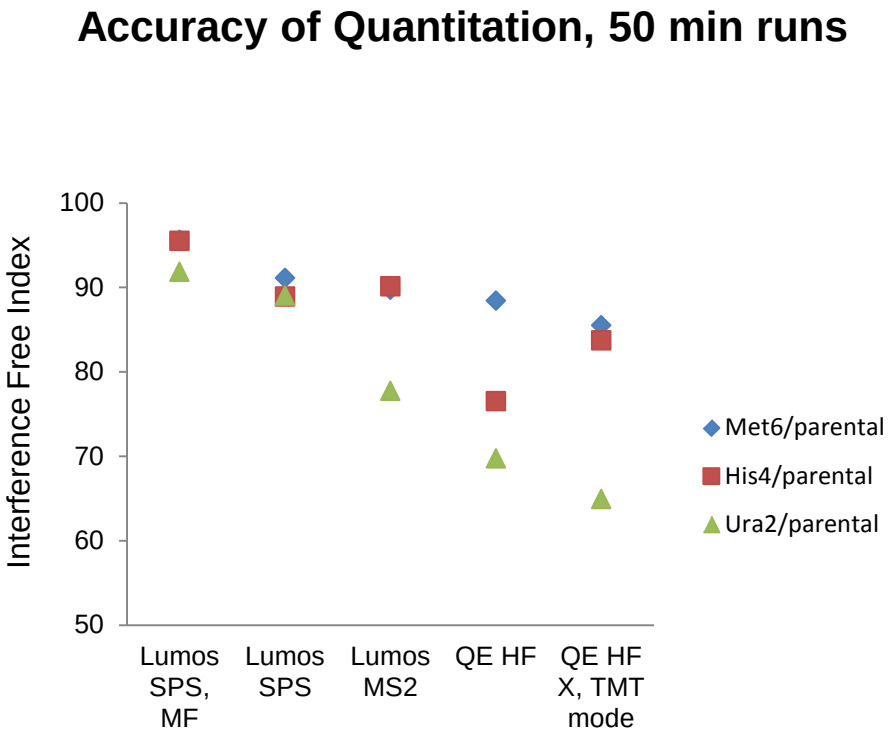
Yeast TKO TMT 11 Standard: QC run Expected Average Instrument Performance





MF= SPS Mass Matches (%), 65%
New feature in PD 2.3

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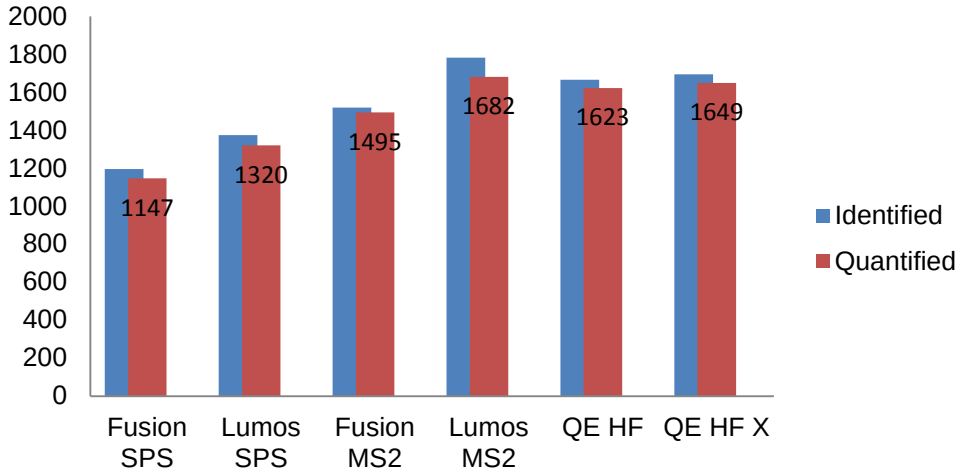
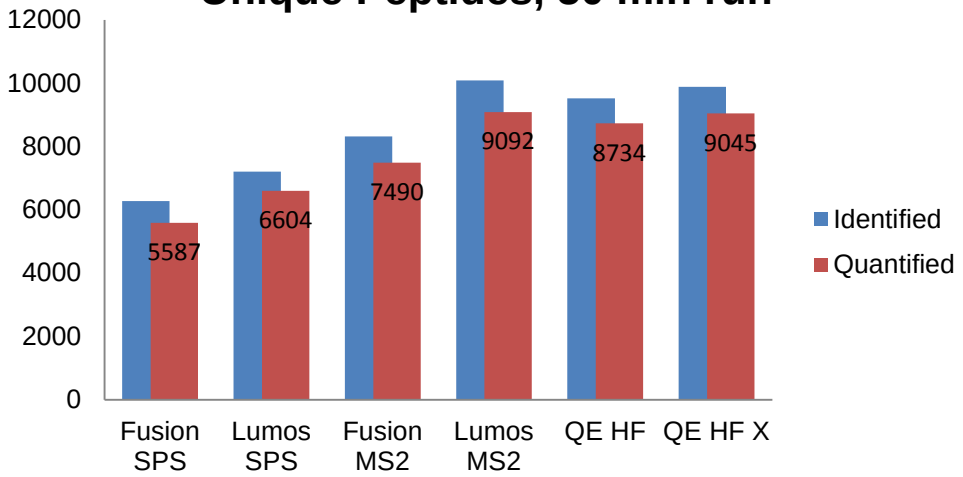
Yeast TKO TMT 11 Standard: QC run Expected Average Instrument Performance

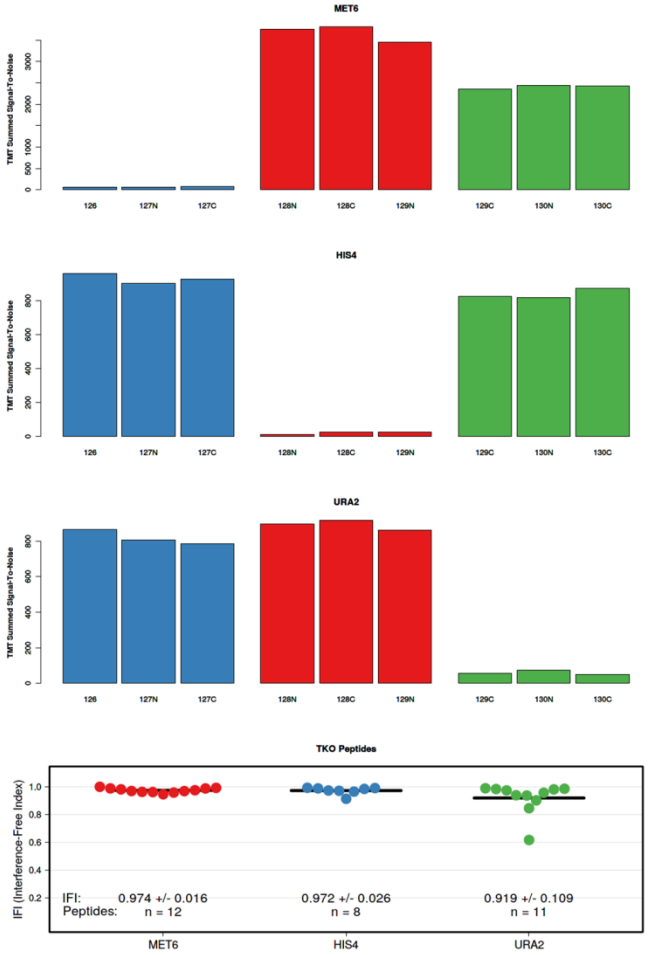
Protein Groups, 50 min run



Unique Peptides, 50 min run

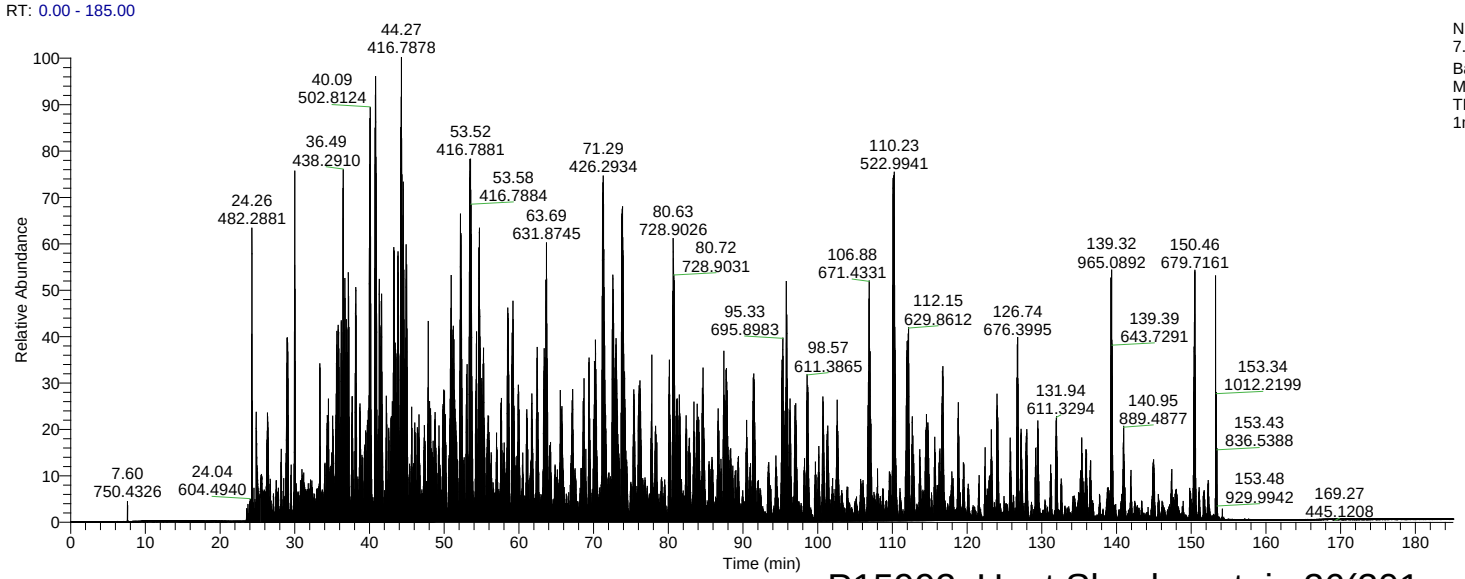


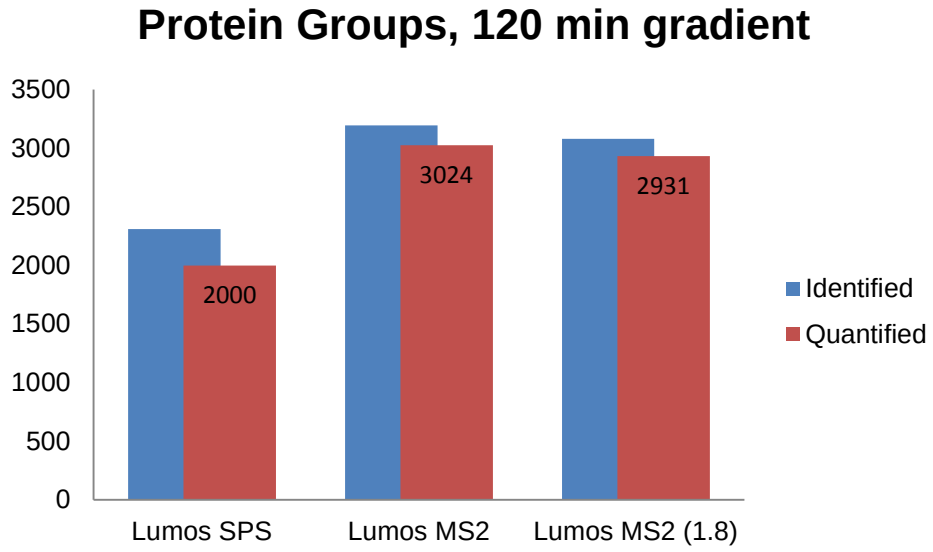
Accuracy of Quantitation, Lumos SPS 50 min run

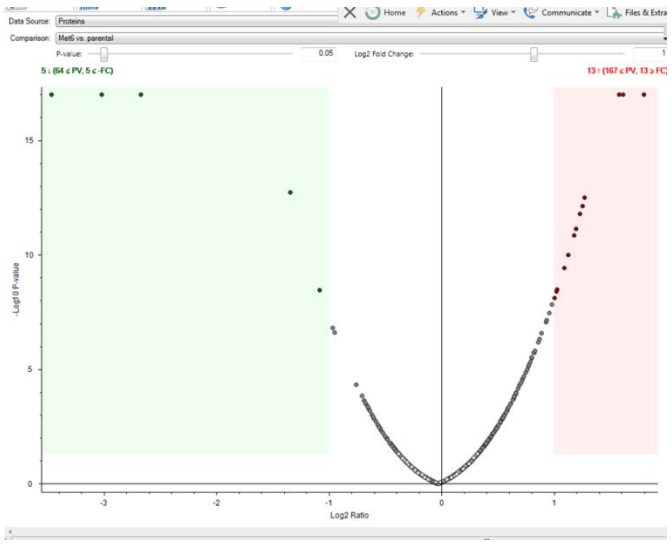


ThermoFisher SCIENTIFIC

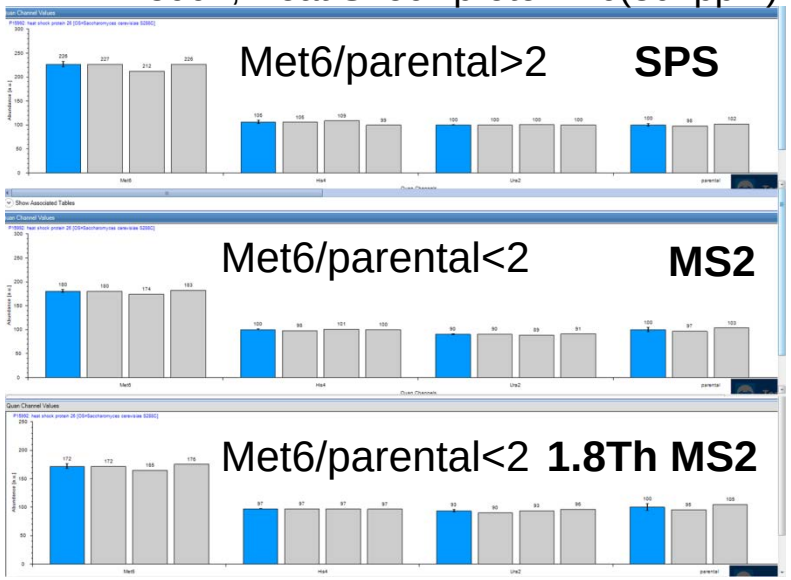
Yeast TKO TMT 11 Standard 2 hour run – Method Development/Optimization

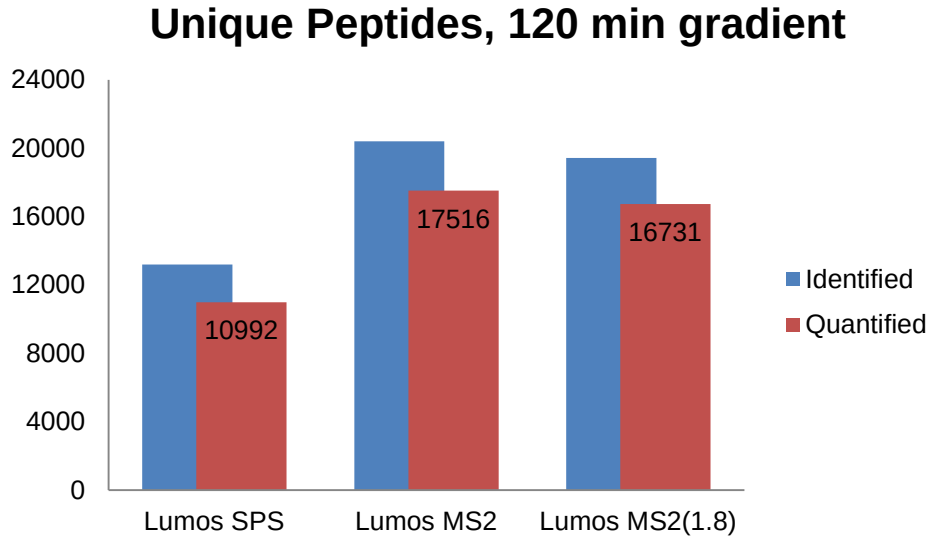






P15992, Heat Shock protein 26(301ppm)





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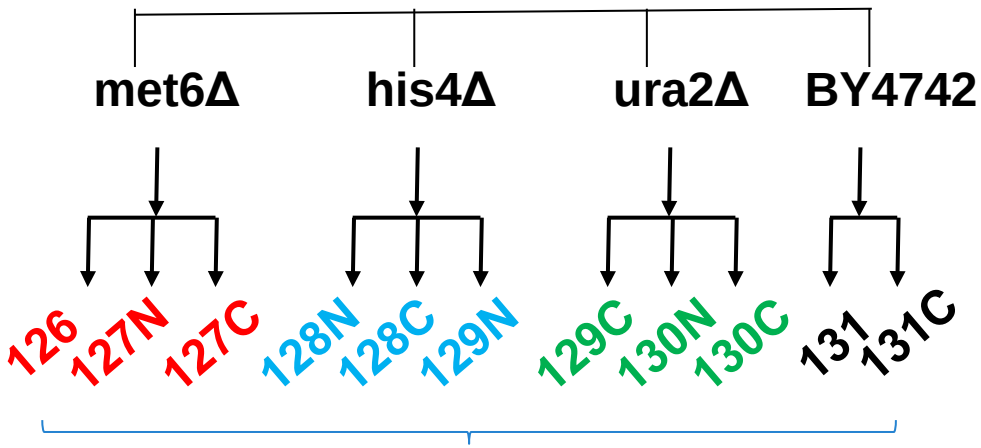
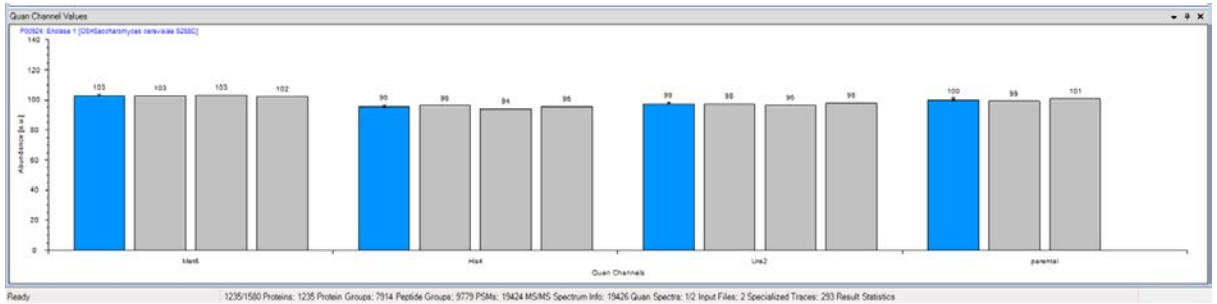
Summary: Product Information- Available October 1

- Excellent tool for LC and mass spec method development
- Excellent QC assay tool for quality assessment of LC and mass spec instrument status
- Provides accuracy, precision and dynamic range assessments for different mass spec strategies
- Optional: TMT labeled Peptide Retention Time Calibration mix (PRTC, yeast heavy isotope peptides) can be spiked in for triggered, multiplexed assay (TOMAHAQ) method development

- **A40938 Pierce TMT11plex yeast digest standard, 20µg**
- **A40939 Pierce TMT11plex yeast digest standard, 5 x 20µg**



Pierce TMT11 yeast TKO standard



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TOMAHAQ Method Development

A Standard Multiplexed Targeted Proteomic Assay Utilizing Isobaric Labels for Evaluation of TOMAHAQ

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Introduction

Triggered by Offset, Multiplexed, Accurate mass, High resolution, and Absolute Quantitation (TOMAHAQ) is a recently introduced targeted proteomics method that combines peptide and sample multiplexing. Establishing TOMAHAQ methods within a lab has proven challenging due to the various formulations of TOMHAQ experiments and lack of a common assay that can be universally implemented.

TOMAHAQ initially combined samples multiplexed with TMT10 and TMT0 labeled synthetic trigger peptides. However, as more labs establish TOMAHAQ assays using available reagents, many questions have been raised:

1) Should trigger peptides be light, heavy, or another proteome;
2) How many peptides should be targeted for method development?;
3) Is method failure due to synthetic peptides, cellular lysate, or instrument performance?

At the core of these questions is the lack of a standard TOMAHAQ assay that can be used to develop and establish TOMAHAQ methods within a laboratory.

Here, we introduce a standard TOMAHAQ assay that utilizes commercially available heavy peptides and whole cell lysate that can be used to create a standard assay for the establishment and evaluation of TOMAHAQ within research laboratories. We combine this assay with recent updates to a freely available program, TomahaqCompanion, which can be used to make TOMAHAQ instrument methods and analyze TOMAHAQ data.

TOMAHAQ + TomahaqCompanion Workflow

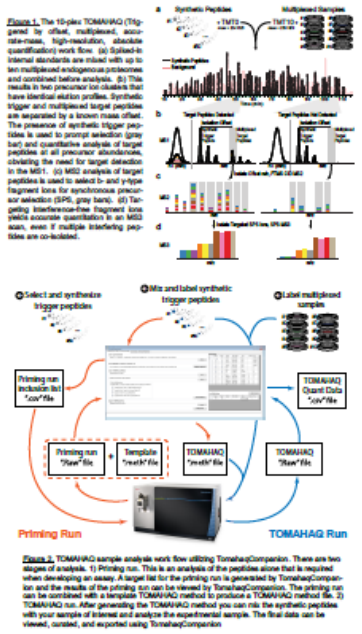
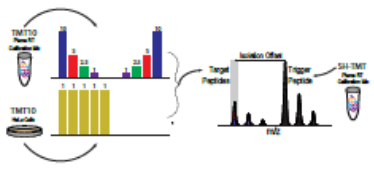


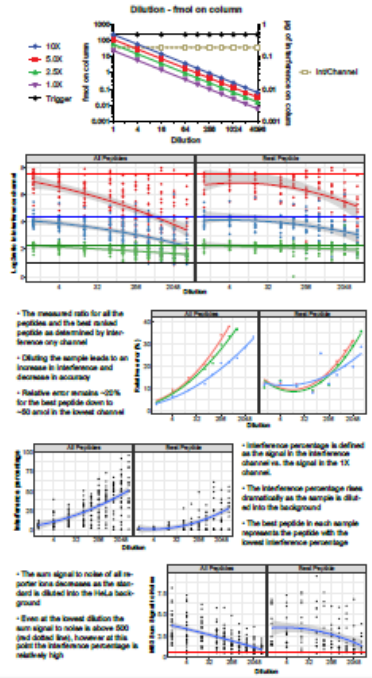
Figure 1. TOMAHAQ sample analysis work flow utilizing TomahaqCompanion. There are two stages of analysis. 1) Priming run. This is an analysis of the peptides alone that is required when developing an assay. A target list for the priming run is generated by TomahaqCompanion and the results of the priming run can be viewed by TomahaqCompanion. The priming run can be combined with a template TOMAHAQ method to produce a TOMAHAQ method file. 2) TOMAHAQ run. After generating the TOMAHAQ method you can mix the synthetic peptides with your sample of interest and analyze the experimental sample. The final data can be viewed, curated, and exported using TomahaqCompanion

A standard mixture for TOMAHAQ evaluation



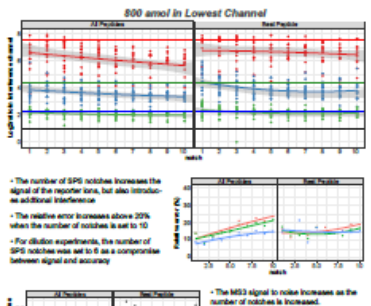
A standard mixture was created to characterize TOMAHAQ methods. The standard mixture comprises Pierce RT calibration (PRTC) peptides, HeLa digest, TMT-10 plex, and SH-TMT reagents. The PRTC peptides were labeled with SH-TMT and spiked into a mixture of HeLa digest (labeled with 5 channels & mixed 1:1) and PRTC peptides labeled with 6 channels of TMT10-plex mixed 10 : 5 : 2.5 : 1 : 0 : 0 : 1 : 2.5 : 5 : 10. To characterize TOMAHAQ sensitivity in a complex background, TMT-10 plex labeled PRTC peptides were mixed at varying amounts relative to the TMT-10 plex HeLa digest background.

Dilution series of standard mixture



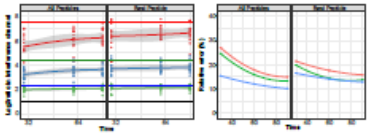
- The measured ratio for all the peptides and the best ranked peptide as determined by interference any channel
- Diluting the sample leads to an increase in interference and decrease in accuracy
- Relative error remains ~20% for the best peptide down to ~60 amol in the lowest channel
- Interference percentage is defined as the signal in the interference channel vs. the signal in the 1X channel.
- The interference percentage rises dramatically as the sample is diluted into the background
- The best peptide in each sample represents the peptide with the lowest interference percentage
- The sum signal to noise of all reporter ions decreases as the standard is diluted into the HeLa background
- Even at the lowest dilution the sum signal to noise is above 500 (red dotted line), however at this point the interference percentage is relatively high

Number of notches



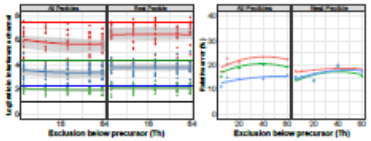
- The number of SPS notches increases the signal of the reporter ions, but also introduces additional interference
- The relative error increases above 20% when the number of notches is set to 10
- For dilution experiments, the number of SPS notches was set to 6 as a compromise between signal and accuracy
- The MS3 signal to noise increases as the number of notches is increased.
- There appears to be a plateau in the amount of signal produced for this sample
- Even for the 1 notch run all peptides have average sum SN greater than 500
- This suggests that a low number of notches can be used
- The interference percentage increases as the number of notches increases
- For this sample, 10 notches still returns an interference percentage less than 20%
- When the best peptide is used the interference percentage is ~10%
- With further SPS filtering this could be improved further

Run length



- Accuracy increases with longer runs as the number of co-eluting peptides is decreased
- The relative error for both the best and all peptides is lowest at 90 min
- For complex mixtures it would be advisable to use longer runs 90 min - 120 min

Precursor range exclusion

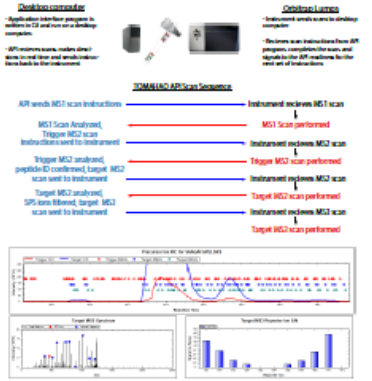


- The area around precursors is often filled with neutral loss peaks from the precursor ions, including interfering ions
- Altering the exclusion window has a minimal effect on the accuracy and relative error, suggesting that in a targeted environment this parameter is less important

TOMAHAQ performed through instrument API

The current implementation of TOMAHAQ through the method editor allows ~150 peptides to be analyzed, but increasing the number of peptides a more advanced TOMAHAQ implementation needed to be created. The instrument API allows a program written in C# to recieve data in real time and send scan instructions. Performing TOMAHAQ through the API will enable:

1. Increase the number of peptides than can be monitored into the thousands
2. Enable more advanced retention time scheduling (elution order scheduling)
3. Allow custom filters to be applied to increase the quantitative accuracy
4. Integration with TomahaqCompanion program (also written in C#)



TOMAHAQ performed through the instrument API was used to analyze the TOMAHAQ standard mix. Each of the peptides was identified and targeted through API directed analysis. The data were visualized using TOMAHAQ companion, which reveals that the API implementation is correctly navigating the complex TOMAHAQ scan structure.

Conclusions and future directions

- Developed a TOMAHAQ standard using components that can be either purchased (Hela, Pierce RT calibration peptides) or acquired (TMT-SH) from Thermo
- Quantifying the best peptide (lowest amount of interference) demonstrates superior quantitative accuracy throughout all conditions tested
- Dilution of TOMAHAQ standard demonstrates quantitative accuracy maintained down to 100 amol in lowest channel (RE < 20% for best peptide)
- Decreasing the number notches increases quantitative accuracy, but limits reporter ion signal in the MS3
- Longer runs increased quantitative accuracy, with the longest run tested (90 min) returning the best quantitative results
- Precursor range exclusion setting does not appear to have a large impact on quantitative accuracy
- TOMAHAQ implemented through the API successfully for TOMAHAQ standard, future work will increase the number of peptides that can be quantified using more advanced RT scheduling and quantitative filters

Acknowledgments

We would like to thank the members of the Microchemistry, Proteomics, and Lipidomics department in Genentech Research & Early Development. We would also like to thank the Thermo research and development team, specifically Derek Bailey and Philip Remes for their work on increasing the number of peptides that can be analyzed through the standard method editor.

References

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